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CUTANEOUS PORPHYRIAS

Clinical and histopathological study

Kaisa Timonen

Academic dissertation

To be presented with the permission of the Medical Faculty of the
University of Helsinki, for public examination
in the Auditorium of the Department of Dermatology and Allergic Diseases
on April 3rd, 2009 at 12 noon.

Helsinki 2009

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ISBN 978-952-92-5116-2 (paperback)
ISBN 978-952-10-5287-3 (pdf)
http://ethesis.helsinki.fi
Helsinki 2009
Yliopistopaino

Motto: Festina lente!

To my patients with porphyria

CONTENTS

SUMMARY

Cutaneous porphyrias are metabolic diseases resulting from the partial deficiency of one of the enzymes in the haem biosynthetic pathway which leads to overproduction of porphyrins in the liver or bone marrow. Because porphyrins are phototoxic compounds, their accumulation in the skin causes photosensitivity. This study was aimed to investigate the characteristics of cutaneous porphyrias.

The prevalence (2.1:100 000, in 2006 n=108) of variegate porphyria (VP) was higher in Finland than elsewhere in European countries due to a founder effect (R152C) and careful screening of the family members. The incidence of VP was estimated at 0.2:1 000 000 based on the number of new symptomatic patients yearly. The prevalence of porphyria cutanea tarda (PCT) was 1.2:100 000 (in 2006 n=63), which is only one fourth of the numbers reported from other European countries. The estimated incidence of PCT was 0.5:1 000 000. Based on measurements of the uroporphyrinogen decarboxylase activity in erythrocytes, the proportion of type II PCT was 49% of the cases. Erythropoietic protoporphyria (EPP) was less common. The prevalence was estimated at 0.8:100 000 (in 2006 n=39) including asymptomatic carriers of a mutation in the ferrochelatase gene. The incidence of EPP was estimated at 0.1:1 000 000.

In VP 37% of the mutation carriers have a manifest disease, and of those 80% have skin symptoms. Of the mutation carriers followed after 1980 (n=57), 30% manifested with skin symptoms with the varying severity. Frequency of skin symptoms only was stable during the follow-up before or after 1980 (22% vs. 21%), but acute attacks only became rare (29% vs. 7%) during the last two decades. The number of patients having both acute attacks and skin symptoms decreased also substantially (22% vs. 11%) but still 30% of the symptomatic patients have both acute attacks and skin symptoms. The most frequent skin symptoms in the patients with VP were fragility (95%) and blistering (46%) of the skin in the backs of the hands. Transient correction of porphyrin metabolism using eight haem arginate infusions within five weeks had no effect on the skin symptoms in three of four patients studied. In one of them skin lesions disappeared transiently.

The patient with homozygous VP is a different entity with severe chronic skin lesions since birth. Sensory polyneuropathy, glaucoma and renal failure developed during the 25-year follow-up without the presence of acute attacks. Four subsequent haem arginate infusions corrected partially porphyrin metabolism for a few days only. The I12T mutation was detected in both of the patient's alleles in the protoporphyrinogen oxidase gene. Lack of skin symptoms and infrequency of acute attacks in the heterozygous patients with I12T mutation indicate a mild phenotype of this mutation at the heterozygous state.

Four mutations (751delGAGAA, 1122delT, C286T, C343T) in the ferochelatase gene were characterised in four of 15 families with EPP. Burning pain (96%) and swelling (92%) of the skin immediately after the sun exposure were the major skin symptoms. More than a 50-fold rise of the protoporphyrin level in erythrocytes predicted severe photosensitivity. Hepatopathy appeared only in one patient.

Clinical manifestations and associated factors of PCT were similar in sporadic and familial types of PCT. In contrast to VP, in the patients with PCT blistering (95%) and fragility (93%) of the skin in the backs of the hands occurred equally. The majority of the patients had one to three precipitating factors, of which alcohol was the most common (78%). Mutations associated with hemochromatosis were frequent (50%), but use of oestrogen (25% of women) and hepatitis B or C

infections (25 %) were less common. Fatty liver disease (67%) and siderosis (67%) were commonly found in liver biopsies (n=21).

The major histopathological change of the sun-exposed skin in VP (n=20), EPP (n=8) and PCT (n=5) was thickening of the vessel walls of the upper dermis suggesting that this is the primary site of the phototoxic reaction in each type of porphyria. The fine structure of the vessel walls was similar in VP, EPP and PCT consisting of the multilayered basement membrane and excess of finely granular substance between the layers which were surrounded by the band of homogenous material. In addition, EPP was characterised by amorphous perivascular deposits extending also to the extravascular space.

The direct immunofluorescence study of the sun-exposed skin revealed constantly homogenous IgG deposits in the vessel walls of the upper dermis in each type of porphyria. IgA, IgM and C3 were present more frequently in EPP than in PCT and VP indicating that the severe vascular damage in EPP results from the abundant leakage of immunoreactants from the circulation. In EPP the excess material around vessel walls consisted of other proteins such as serum amyloid protein, and kappa and lambda light chains in addition to the basement membrane constituents such as collagen IV and laminin. These results suggest that the alterations of the vessel walls are a consequence of the repeated damage and the repairing process in the vessel wall.

The microscopic alterations could be demonstrated even in the normal looking but sun-exposed skin of the patients with EPP during the symptom-free phase suggesting that vascular change can be chronic. The presence of microscopical changes in those patients with VP who had never experienced skin symptoms suggests that photodamage at the level of dermal vessels can be a subclinical process. The stability of vascular changes in the patients with PCT after treatment indicates that circulating porphyrins are not important for the maintenance of the changes.

LIST OF ORIGINAL PUBLICATIONS

I Timonen K, Niemi K-M, Mustajoki P, Tenhunen R. Skin changes in variegate porphyria. Clinical, histopathological and ultrastructural study. Arch Dermatol Res 282:108-114, 1990.

II Timonen K, Mustajoki P, Tenhunen R, Lauharanta J. Effects of haem arginate on variegate porphyria. Br J Dermatol 123:381-387, 1990.

III Kauppinen R, Timonen K, von und zu Fraunberg, M, Laitinen E, Ahola H, Tenhunen R, Taketani S, Mustajoki P. Homozygous variegate porphyria: 20 y follow-up and characterization of molecular defect. J Invest Dermatol. 116:610-613, 2001.

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V Timonen K, Kariniemi A-L, Niemi KM, Teppo A-M, Tenhunen R, Kauppinen R. Vascular changes in erythropoietic protoporphyria. Histopathologic and immunohistochemical study. J Am Acad Dermatol. 43:489-497, 2000.

VI Timonen K, Niemi K-M, Mustajoki P. Skin morphology in porphyria cutanea tarda does not improve despite clinical remission. Clin Exp Dermatol 16:355-358, 1991.

In addition, unpublished data is presented.

ABBREVIATIONS

ALA	Aminolaevulinic acid
ALAS	5-Aminolaevulinic acid synthase
BMZ	Basement membrane zone
CEP	Congenital erythropoietic porphyria
C3	Complement 3
DNA	Deoxyribonucleic acid
DIF	Direct immunofluorescence examination
EPP	Erythropoietic protoporphyria
EM	Electron microscopic examination
FECH	Ferrochelatase
HFE gene	Hemochromatosis associated gene
Ig	Immunoglobulin
LM	Light microscopic examination
MIM	Mendelian Inheritance in Man (Stedman's Medical Dictionary)
PAS	Periodic acid-Schiff
PBG	Porphobilinogen
PCT	Porphyria cutanea tarda
PPOX	Protoporphyrinogen oxidase
UROD	Uroporphyrinogen decarboxylase
VP	Variegate porphyria

INTRODUCTION

Porphyrias are a group of metabolic diseases due to an inherited deficiency of the haem biosynthesis. Each of seven porphyrias is related to one of the eight enzymatic steps in the haem biosynthetic pathway (Figure 1). The genes which encode the enzymes for haem production have been characterised and several mutations in these genes have been identified. A decreased enzyme activity results in overproduction of porphyrins and their precursors either in the bone marrow as in erythropoietic porphyrias or in the liver as in hepatic porphyrias (Anderson et al. 2001). Excess of porphyrins or their precursors is responsible for photosensitivity and acute attacks, which are the two major clinical manifestations of porphyria. Photosensitivity occurs in all subtypes of porphyrias except in delta-aminolaevulinic acid (ALA)-dehydratase deficiency (MIM 125270) and acute intermittent porphyria (AIP; MIM 176000). Circulating porphyrins enter the skin where they interact with the light energy resulting in a phototoxic reaction. Thus, cutaneous porphyrias are a unique group of photosensitivity diseases caused by the endogenous agent. Acute attacks are related to an overproduction of porphyrin precursors in the liver induced by several precipitating factors and they are manifested by autonomic and peripheral neuropathy and central nervous system injury.

The skin symptoms of porphyrias manifest clinically as an immediate or chronic photosensitivity depending on the type of porphyrin which accumulates in the skin. In contrast to other photodermatoses in which ultraviolet radiation is usually responsible for photosensitivity in porphyria visible light induces the skin symptoms due to the distinctive light absorbing spectra of porphyrin molecules (Moore et al. 1987). Toxic properties of porphyrins may also entail hepatobiliary disorders in cutaneous porphyrias as in porphyria cutanea tarda (PCT; MIM 176100) and erythropoietic protoporphyria (EPP; MIM 177000)(Bloomer et al. 2006).

Each subtype of porphyria with clinical symptoms has characteristic features of the blood porphyrin profiles and the excretion of porphyrin and their precursors. Thus, biochemical analyses provide a basic instrument for the diagnostics and are mandatory to confirm the correct diagnosis in the symptomatic phase (Moore et al. 1987; Elder et al. 1990). Because the traditional biochemical methods are not sensitive or specific enough to find asymptomatic family members with porphyria, a mutation screening may be the only way to detect these individuals (Kauppinen 2005).

1. CLASSIFICATION OF PORPHYRIAS

Porphyrias are classified as erythropoietic or hepatic according to the tissue where the excess of porphyrins or their precursors are predominantly synthesized (Table 1) (Anderson et al. 2001). Porphyrias are also divided into acute and cutaneous porphyrias depending on the type of symptoms. Hepatic porphyrias manifest themselves by the acute attacks except PCT. Hereditary coproporphyria (HCP; MIM 121300) and variegate porphyria (VP; MIM 176200) are characterised by both skin symptoms and acute attacks. Acute attacks are lacking in erythropoietic porphyrias.

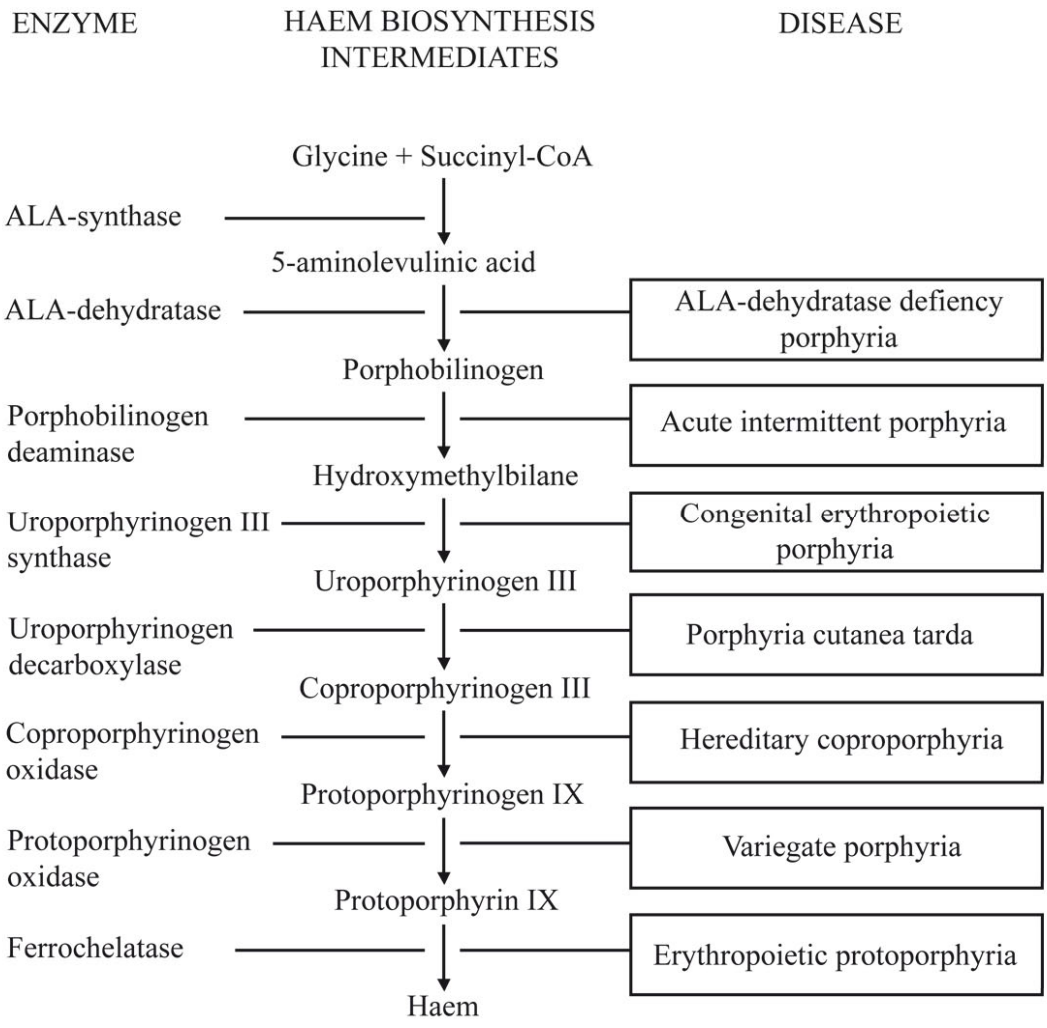


Figure 1. The enzymes in the haem biosynthetic pathway and the corresponding type of porphyria.

2. INHERITANCE AND MOLECULAR GENETICS OF PORPHYRIAS

Porphyrias are inherited as an autosomal dominant fashion except extremely rare ALA-dehydratase deficiency and congenital erythropoietic porphyria (CEP; MIM 263700). The latter is known also as Günther's disease, and it has an autosomal recessive pattern of inheritance (Table 1) (Anderson et al. 2001). In each autosomally dominantly inherited porphyria, homozygous or compound heterozygous disorders have been identified. They are very rare and manifest usually with more severe symptoms in the early childhood (Hift et al. 1993).

The inheritance of PCT is more complex. The disorder can present as a sporadic form (type I) or familial form (types II and III, Table 1) (Elder et al. 1989). In type I, which is the most common form of PCT, no defects in the uroporphyrinogen decarboxylase (UROD; EC 4.1.1.37) gene are currently known and the enzyme deficiency is probably limited to the liver (Elder et al. 1978). In the familial forms mutations in the UROD gene have been identified in type II which is characterised by low enzyme activity measured in erythrocytes or other cell lines (Elder et al. 1989; Bygum et al. 2003; Poblete-Gutierrez et al. 2004; Mendez et al. 2007). So far, the genetic background is ambiguous in the type III, in which the enzyme activity in erythrocytes is normal as in type I PCT despite positive family history (Elder et al. 1989; Mendez et al. 2007).

The genes for all enzymes in the haem biosynthesis have been cloned and sequenced and their chromosomal localization is known (Table 1). In each type of porphyria, numerous mutations have been identified including missense, nonsense, splicing defects as well as deletions and insertions (See for more detailed information in Human Gene Mutation database: http://www.hgmd.org). Family-specificity and heterogeneity of mutations and an incomplete penetrance are characteristic features of all types of porphyria (Anderson et al. 2001). Although a mutation in an allele results in the loss of the enzyme activity, a residual 50% or less of the total activity measured in patients is usually sufficient for the normal metabolism of haem.

The penetrance of different porphyrias varies, but in AIP and VP around 40-60% of the gene carriers have clinical manifestations (von und zu Fraunberg et al. 2002; Hift et al. 2004b; von und zu Fraunberg et al. 2005). The penetrance in other cutaneous porphyrias based on mutation analyses is not known.

Since the presence of a mutation does not necessarily result in a porphyric phenotype, additional genetic and environmental factors are usually required for clinical or biochemical manifestations. Such genetic factors may include polymorphism of the cytochrome P450 genes related to a metabolism of drugs or other porphyrinogens influencing the manifestation of acute porphyrias (von und zu Fraunberg et al. 2005) and PCT (Christiansen et al. 2000; Gardlo et al. 2003). Moreover, polymorphism in the ferrochelatase (FECH) gene affects the phenotype of EPP (Gouya et al. 2002), and mutations in the hemochromatosis gene (*HFE*) are associated with PCT (Bulaj et al. 2000). Intake of drugs, alcohol and oestrogen preparations in addition to hepatitis virus infections are examples of extrinsic precipitating factors in hepatic porphyrias (Anderson et al. 2001).

Despite the heterogeneous molecular background of porphyrias, the clinical and biochemical manifestations are quite uniform in these patients. To date, genotype-phenotype correlation has been reported in CEP, VP, EPP, and AIP (Warner et al. 1992; Minder et al. 2002; von und zu Fraunberg et al. 2002; von und zu Fraunberg et al. 2005), but this relationship has not been confirmed in all patients series (Whatley et al. 1999; Lamoril et al. 2001).

Table 1. Classification and characteristics of porphyrias.

Classification	Deficient enzyme	Inheritance	Chromosomal location	Current number of mutations[1]	Main clinical manifestation
Erythropoietic					
Congenital erythropoietic porphyria	Uroporphyrinogen III synthase	Autosomal recessive	10q26	38	Photosensitivity Haemolytic anaemia
Erythropoietic protoporphyria	Ferrochelatase	Autosomal dominant	18q21.3	99	Photosensitivity
Hepatic					
ALA dehydratase deficiency porphyria	ALA dehydratase	Autosomal recessive	9q34	11	Neuropathy
Acute intermittent porphyria	Porphobilinogen deaminase	Autosomal dominant	11q24	277	Acute attacks
Hereditary coproporphyria	Coproporphyrinogen oxidase	Autosomal dominant	3q12	44	Acute attacks Photosensitivity
Variegate porphyria	Protorphyrinogen oxidase	Autosomal dominant	1q22	137	Acute attacks Photosensitivity
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase		1p34		
Type I, sporadic	Activity decreased in liver	Pattern of inheritance not known			Photosensitivity Hepatopathy
Type II, familial	Activity decreased in all cells	Autosomal dominant		63	Photosensitivity Hepatopathy
Type III, familial	Activity decreased in liver cells	Autosomal dominant			Photosensitivity Hepatopathy

[1]October 2007 http://www.hgmd.org

Development of animal models has provided new tools to study molecular background, pathogenesis and treatment of human diseases. Unfortunately, the PBGD deficient mice resemble the homozygous type of acute intermittent porphyria with chronic neuropathy but no acute attacks, and biochemical changes could be activated only by administration of barbiturates (Lindberg et al. 1996). Furthermore, the mouse model for VP manifested the biochemical abnormalities but no clinical symptoms (Medlock et al. 2002). Photosensitivity and liver failure were observed (Tutois et al. 1991; Meerman et al. 1999) and skin symptoms could be relieved by the gene therapy in the mouse model for EPP (Richard et al. 2004). Heterozygous UROD deficient mice were more vulnerable to iron load and hepatopathy but no cutaneous symptoms were detected (Phillips et al. 2001).

3. HAEM BIOSYNTHESIS

Haem (iron protoporphyrin IX) is a tetrapyrrole containing a central iron ion (Anderson et al. 2001) and it is crucial for the life. Even though it is synthesised in all mammalian cells, around 85% of haem, which is essential for haemoglobin synthesis, is produced in the maturating erythrocyte precursors in the bone marrow (Anderson et al. 2001). Haem also participates in the regulation of the globin synthesis to optimize its level (Chen 2007). The liver is another major source of haem. Most of the hepatic haem is utilized in the synthesis of cytochrome P-450 enzymes and for many essential cellular haemoproteins which participate in energy supply and respiration.

The initial and terminal three enzymes of the haem biosynthesis are located inside the mitochondrion, while the other four are in the cytosol (Figure 2). During the first step, the condensation of eight molecules of glycine and eight molecules of succinyl coenzyme A is catalyzed by ALA-synthase (ALAS) to form ALA. Subsequently, two molecules of ALA are condensed to a monopyrrole porphobilinogen (PBG) by ALA-dehydratase. Then, four PBG molecules are condensed by the cytosolic PBGD to form a tetrapyrrole intermediate - hydroxymethylbilane. During the fourth step, the tetrapyrrole is sealed by uroporphyrinogen III synthase to form uroporphyrinogen III. Normally less than 1% of hydroxymethybilane is converted non-enzymatically to uroporphyrinogen I, but this route is activated in the deficiency of uroporphyrinogen III synthase associated with congenital erythropoietic porphyria (CEP).

In the fifth step, the four carboxylic groups of the acetic acid chains in uroporphyrinogen are removed step by step by UROD (EC 4.1.1.37) resulting in hepta-, hexa- and pentacarboxylic intermediates before the formation of coproporphyrinogen III. During the next stage, coproporphyrinogen oxidase which is located in the intermembrane space of mitochondria decarboxylates two of the four propionic groups in coproporphyrinogen III to two vinyl groups yielding protoporphyrinogen IX.

At the penultimate stage oxidation of protoporphyrinogen IX to protoporphyrin IX is catalyzed by protoporphyrinogen oxidase (PPOX; EC 1.3.3.4). Six hydrogen atoms are removed from the porphyrinogen nucleus. PPOX is a homodimeric flavoprotein and is located on the outer surface of the inner mitochondrial membrane in this reaction (Dailey et al. 2005). Iron is inserted into protoporphyrin to form haem in the terminal step. The reaction is catalysed by FECH (EC 4.99.1.1) which is also known as haem synthase. The human FECH has a homodimeric structure and it is boud to the matrix side of the inner mitochondrial membrane (Dailey et al. 2005).

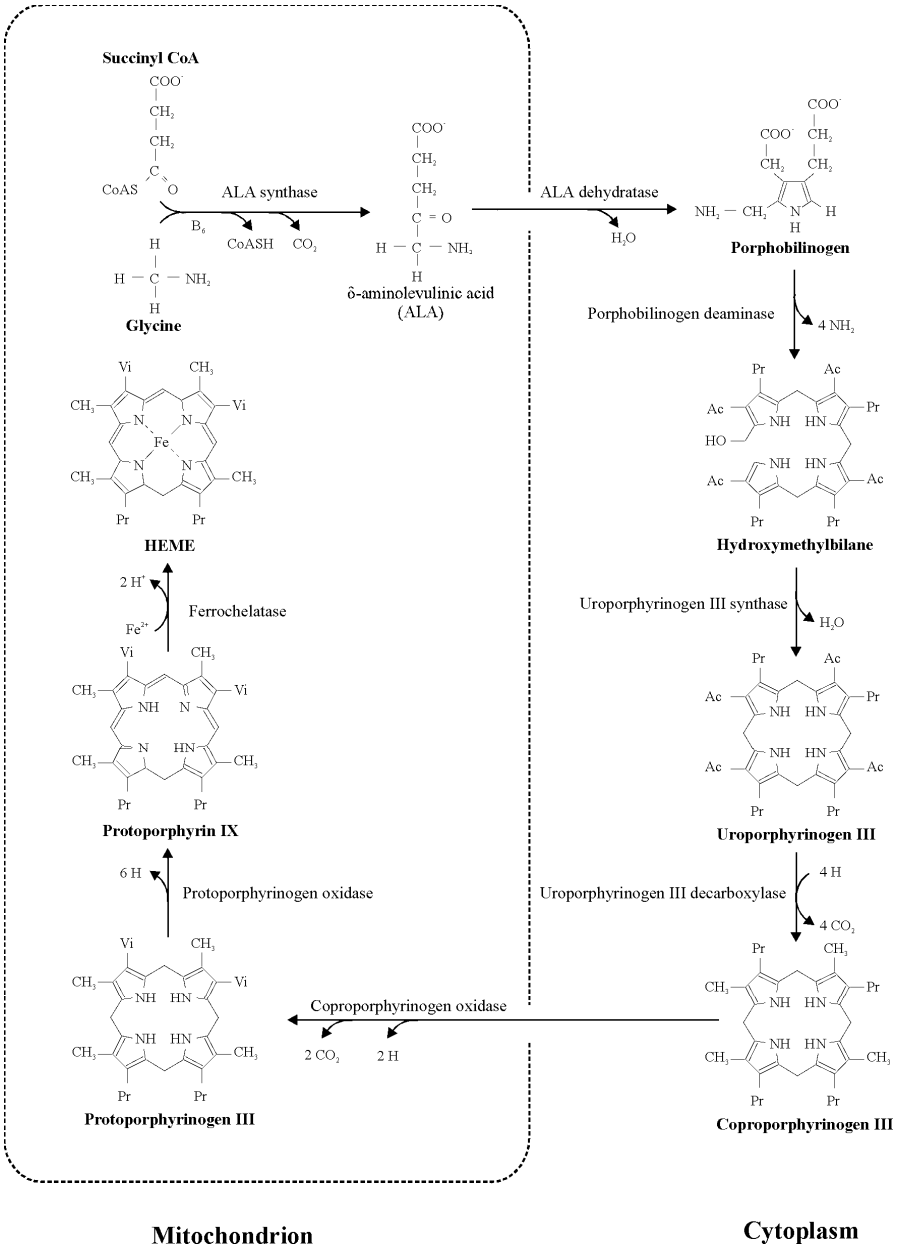


Figure 2. The enzymatic steps of haem biosynthesis

Haem biosynthesis is regulated by two isoforms of ALAS coded by two separate genes (Bishop et al. 1990). The housekeeping isoform (ALAS1) presents in the liver and in the other non-erythroid tissues. The synthesis is repressed by haem and its activity is enhanced by various drugs, alcohol and other chemicals (Sassa and Nagai 1996). Erythroid-specific isoform (ALAS2) is expressed in erythroid cells only. In contrast to ALAS1, the synthesis is upregulated by the increased haem concentration and by the availability of iron (Sadlon et al. 1999; Zoller et al. 2002).

Determination of three-dimensional crystal structures of the recombinant enzymes and characterisation of mitochondrial targeting signals of enzymes have allowed studies of structural and functional changes in mutants and their intracellular transport mechanisms (Ajioka et al. 2006). Further studies will clarify the pathogenetic mechanisms of these disorders at the cellular and molecular level.

4. PHOTOSENSITIVITY IN PORPHYRIAS

The photosensitizing properties of porphyrins were first demonstrated in 1913 by a physician Meyer-Betz, who injected himself intravenously haematoporphyrin (Meyer-Betz 1913). Shortly thereafter he had marked erythema and oedema of the skin after the sun exposure resembling skin symptoms of EPP and later on chronic photosensitivity similar to PCT.

Porphyrins are phototoxic compounds due to their molecular configuration (Figure 3). An extensive double-bond structure of tetrapyrrole ring and one of several ions at the centre render porphyrin strong absorbers of the light energy (Poh-Fitzpatrick 1986). The most important absorption bands of porphyrins lie in the visible region between 400–410 nm (Soret band) and to smaller extent between 500-650 nm (Figure 4). The action spectrum studies performed in patients with porphyric skin symptoms have shown similarly that a peak response eliciting cutaneous photosensitivity lies at around 400 nm and to a lesser extent between 500 and 600 nm (Magnus 1961; Rimington et al. 1967).

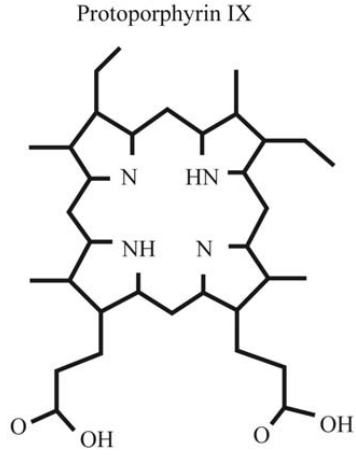
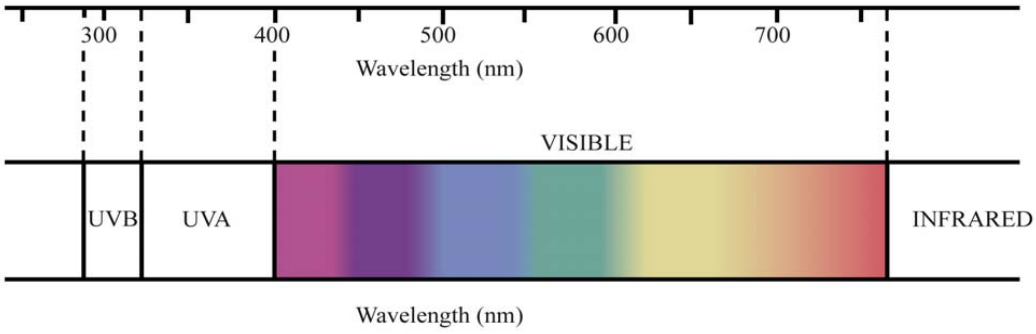


Figure 3. The structure of protoporphyrin IX.

Figure 4. The spectrum of the solar radiation.



Absorption of photon converts stable ground–state porphyrin molecule to the singlet or triplet excited state which is unstable of character (Figure 5). Porphyrin sensitised photoreaction is classified into two types. In type I reaction, transfer of a hydrogen atom or electron from the singlet excited state porphyrin to molecular oxygen produces free radicals which cause cellular damage. The singlet excited state may return to ground status by emission of typical red fluorescence or it may be converted to the excited triplet state porphyrin.

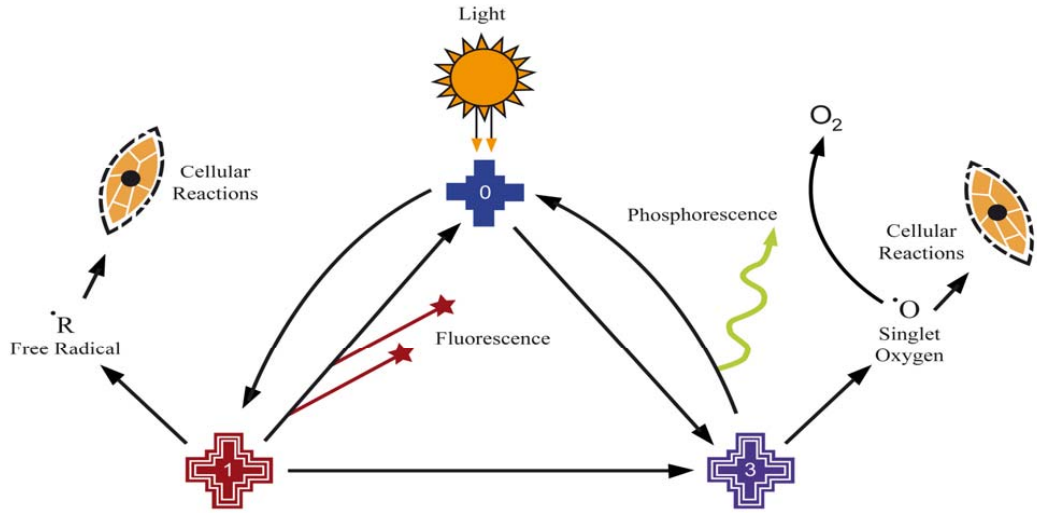


Figure 5. Porphyrin-induced phototoxic reaction.

Type II photoreaction arises from the triplet excited state molecule which generates highly reactive singlet oxygen. Excited oxygen may react with hydrogen to form peroxides which are destructive for cellular structures such as cell membranes (Spikes 1983; Poh-Fitzpatrick 1986). In EPP lipid peroxidation of red cell membrane caused by long wave ultraviolet exposure could be responsible for photohaemolysis which results in releasing the protoporphyrin into the circulation

(Goldstein and Harber 1972; Brun et al. 1990). Recently, the generation of oxygen radicals has been demonstrated in vivo during light irritation in the skin of protoporphyric mouse (Takeshita et al. 2004).

Photosensitized porphyrins are also capable of inducing other cellular events which may augment the tissue damage. Mast cell degranulation (Glover et al. 1990), complement activation (Gigli et al. 1980) and increase of collagen synthesis by fibroblasts have been demonstrated in *vivo* and in *vitro* experiments (Varigos et al. 1982). Uroporphyrin I combined with the long wave UVA radiation augments the synthesis of matrix metalloproteinases (MMP-1 and MMP-3) in fibroblast culture to a greater degree than by UVA radiation alone but has no effect on the synthesis of the major inhibitor of metalloproteinases TIMP-1 (Herrmann et al. 1996). These results suggest that the unbalanced synthesis of metalloproteinases may contribute to the injury of the connective tissue leading to blistering and photoaging of the skin in PCT (Herrmann et al. 1996). Furthermore, an increased proteolytic activity of MMP-2 (72-kDa type IV collagenase) and MMP-3 (stromelysin-1) has been demonstrated in the blister fluids of the patients with PCT supporting the implication of metalloproteinases in the blister formation (Herrmann et al. 1996).

Photosensitization *via* porphyrins produces two different clinical manifestations: acute skin reactions characterised by a burning sensation, oedema and erythema immediately after the sun exposure, and chronic skin lesions including blisters and erosions aggravated by mechanical trauma in continuously sun-exposed areas (Murphy 1999). The first one occurs in EPP and CEP. Hepatic cutaneous porphyrias, HCP, PCT and VP are characterised by a chronic type of photosensitivity. CEP manifests with the most severe photosensitivity and is characterised by burning reaction with vesicles, bullae and erosions resulting in scarring and mutilations especially on the face and hands in addition to erythrodontia and severe anaemia (Murphy 2003).

The acute or chronic types of skin symptoms have been explained by different intracellular localization and solubility of porphyrins in the aqueous and lipid phases (Sandberg et al. 1982). Protoporphyrin has high affinity for lipoproteins in cellular membranes and mitochondria because of its lipophilic nature. Uroporphyrin is water soluble and accumulates in lysosomes. Thus, the clinical manifestations may be associated with the intracellular site of the primary photodamage.

Porphyrins vary in their phototoxicity. It has been shown experimentally that protoporphyrin IX is the most potent photosensitiser (Menon et al. 1990). Coproporphyrin III is less efficient than protoporphyrin IX and uroporphyrin III is the least potent. However, this is in contrast to the clinical experience that the most severe photosensitivity occurs in CEP with a drastic increase of accumulation of uroporphyrin I (Murphy 2003).

Photodynamic therapy used for the treatment of various malignant and premalignant tumors of the internal organs and the skin, is a clinical application of phototoxicity of porphyrins (Wiedmann and Caca 2004; Babilas et al. 2005). Lethal cell damage is achieved by a systemic administration of haematoporphyrin derivatives or by topical application of porphyrin precursor ALA, which is converted intracellulary to protoporphyrin IX, and by subsequent irradiation with an appropriate light source.

Absence of photosensitivity in AIP is related to the fact that porphyrin precursors (ALA, PBG) which accumulate in this disorder are not phototoxic. Although acute attacks are accompanied by high urinary excretions of uroporphyrin I and III and spectrofluorometric assay of plasma shows fluorescence maximum at 619 nm due to uroporphyrins as in PCT, skin symptoms are not present. The presence of porphyrin in the skin is probably too transient to produce skin symptoms.

REVIEW OF LITERATURE

1. VARIEGATE PORPHYRIA

VP was first identified in the 1930s (van der Bergh and Grotepass 1937). It results from the mutations in the PPOX gene which encodes the last second enzyme in the haem biosynthesis. As a consequence of a mutation the total cellular activity of PPOX is decreased approximately to 50% of that of normal which results in overproduction of protoporphyrin and coproporphyrin in the circulation. In addition, porphyrin precursors, PBG and ALA, are overproduced in the liver during acute attacks (Mustajoki 1978).

1.1. Prevalence of VP

The highest prevalence of VP has been reported from South Africa among the Afrikaners (1:300) (Dean 1971). According to later calculations 20 000 South Africans may carry the gene defect of VP (Kirsch et al. 1998). Genealogical studies have shown that they are descendents from one of the first Dutch settlers from the 17th century (Dean 1971). In the earliest study from Finland the prevalence of VP has been estimated 1.3:100 000 in population above the age of 15 years (Mustajoki 1980). In the more recent study the prevalence of VP was approximately 1.9:100 000 in the whole Finnish population (von und zu Fraunberg et al. 2002). VP is less common in other countries. The estimated prevalence of VP in the United Kingdom is 0,5:100 000 (Elder et al. 1997), in Sweden 1:100 000 (Wiman et al. 2003b) and in Argentina 1:600 000 (Parera et al. 2003). Among acute porphyrias, VP is less common than AIP except in South Africa and in Chile (Armas et al. 1992; Nordmann and Puy 2002).

1.2. Inheritance and molecular genetics of VP

VP is inherited by autosomal dominant trait. The gene coding PPOX has been characterised (Nishimura et al. 1995; Taketani and Fujita 1995) and mapped to chromosome 1q22 (Roberts et al. 1995). The genomic sequence is spread over 13 exons (12 coding and one non-coding) and spans 5,5 kb (Puy et al. 1996) The first mutations of the PPOX gene were reported in 1996 (Deybach et al. 1996; Meissner et al. 1996; Warnich et al. 1996). By October 2007 137 different mutations have been reported in the PPOX gene in VP patients (Human Gene Mutation Database:http://www.hgmd.org). The mutations have usually been family specific. A founder effect may affect a local prevalence and for example in South Africa 95% of the patients have inherited the same R59W mutation from a common Dutch ancestor (Meissner et al. 1996; Warnich et al. 1996). In Chile, where VP is also the most common type of acute porphyria (Armas et al. 1992), the founder mutation in the PPOX gene (1239delTACAC) has been identified (Frank et al. 2001). Eleven mutations in the PPOX gene have been identified in 22 Finnish VP families to date (von und zu Fraunberg et al. 2002; R.Kauppinen, personal communication). One major mutation (R152C) has been identified in 11 families (50%) (von und zu Fraunberg and Kauppinen 2000).

1.3. Clinical features of VP

Based on different patient series, 20-50% of gene carriers have a clinically overt disease (Kirsch et al. 1998; von und zu Fraunberg et al. 2002; Hift et al. 2004b). Many environmental factors and other modifying genes play a crucial role in the phenotype of VP. Sex hormones also affect clinical manifestations, since acute porphyrias do not manifest before puberty. The majority of patients are 20-40 years old at the onset of the disease (Mustajoki 1980). VP manifests by occasional acute attacks, which may be life-threatening, if undiagnosed, and by a chronic type of photosensitivity. These symptoms occur independently from each other (Mustajoki and Koskelo 1976; Day 1986).

1.3.1. An acute attack

An acute attack, which is indistinguishable from that of AIP and HCP, is usually precipitated by both exogenous and endogenous factors. The most common precipitating factors include drugs, infections, alcohol and fasting (Kauppinen and Mustajoki 1992; Anderson et al. 2001). These factors induce ALAS and consequently haem biosynthesis resulting in increased excretion of porphyrins and their precursors from the liver into the circulation. The recurrent attacks are rare in VP and the menstrual cycle precipitates acute attacks less commonly in comparison with AIP (Kirsch et al. 1998).

The characteristic findings during an acute attack are presented in Table 2. The initial symptoms include abdominal pain, constipation and vomiting accompanied by tachycardia and hypertension, which are signs of autonomic neuropathy. The peripheral neuropathy is another major feature and usually manifests as the pain in the extremities and/or the back. The acute attack may lead to lethal respiratory paralysis or cardiac arrest.

Table 2. Frequency (%) of symptoms of an acute attack in VP

	N=18[1]	N=100[2]	N=23[3]
Abdominal pain	95	98	95
Tachycardia	90	80	35
Constipation	80	60	30
Neuropathy	60	60	<10
Vomiting	65	70	55
Hypertension	60	60	50
Respiratory muscle paralysis	40	25	7
Epileptic seizures	25	25	5

[1]Mustajoki (1980); [2]Eales et al. (1980); [3]Kirsch et al. (1998)

The main hypotheses of the pathogenesis of neuropathy during an acute attack are related to direct neurotoxicity of ALA, and the local deficiency of haem in neural tissue (Anderson et al. 2001; Meyer et al. 1998). The studies in the transgenic PBGD mouse suggest that multiple mechanisms are involved including ALA interaction with GABA receptors and changes in tryptophan metabolism (Meyer et al. 1998). Liver transplantation in patients with AIP and VP, which has resulted in normalization of porphyrin metabolism and clinical symptoms, has demonstrated the importance of the liver as a main source of accumulation of porphyrin metabolites (Seth et al. 2007). However, the details of pathogenetic events of an acute attack still remain ambiguous.

1.3.2. Skin symptoms

In contrast to acute attacks, no specific factors except solar exposition are known to induce skin symptoms. The skin manifestations of VP are presented in Table 3. The characteristic skin symptoms include blisters, erosions and excessive skin fragility of the sun-exposed areas, usually on the backs of the hands and the face, and less commonly on the arms, legs and feet (Mustajoki 1980; Muhlbauer et al. 1982; Day 1986). The lesions heal slowly with crusting, scarring and milia formation. The development of skin lesions requires repeated sun exposure and the symptoms persist without the direct sun exposure. The symptoms are usually present during the summer time worsening in the late summer. A half of the 17 Finnish VP patients with skin symptoms reported no seasonal variation in the skin fragility (Mustajoki 1980). The patients complained mainly of the mild skin fragility. Other chronic skin symptoms of VP include hypertrichosis and hyperpigmentation on the face skin (Corey et al. 1980; Muhlbauer et al. 1982; Day 1986). All skin manifestations in VP are indistinguishable clinically from those of PCT and HCP.

Table 3. Skin manifestations in VP

Skin fragility of the sun-exposed skin (back of the hands and face)
Erosions and blisters in the sun-exposed skin (back of the hands and face)
Scarring and hypopigmentation on the old skin lesions
Milia formation on the old skin lesions
Hypertrichosis on the face skin
Hyperpigmentation on the face skin

Figure 6. Bulla and erosions on the dorsal side of the hand in a patient with VP (on the courtesy of the patient)

The frequency of skin symptoms reported in different patient series is quite constant. In the series including 103 patients from France and United Kingdom 80% of symptomatic VP patients reported skin symptoms, and 20% of them had also acute attacks (Whatley et al. 1999). Of the 28 VP patients from one South African family with R59W mutation, 40% of the cases has experienced photosensitivity (Hift et al. 2004b). In the Finnish series, which included 103 VP patients having various mutations, the frequency of photosensitivity was 76% in symptomatic and 40% in all VP patients diagnosed either with mutation screening or with biochemical analyses (von und zu Fraunberg et al. 2002). In an earlier Finnish study the frequency of photosensitivity was 45% of all VP patients (Mustajoki 1980) indicating that proportion of patients with skin symptoms has not changed in Finland and is similar to the frequency in other countries.

1.4. Histopathology of the skin in VP

Of South African patients with VP, six subjects have been reported to have typical skin fragility in the sun-exposed areas, and histological examination revealed lipoid proteinosis-like changes in the skin (Findlay et al. 1966). It has been shown in the light microscopy (LM) that these areas demonstrate periodic acid-Schiff (PAS)-positive hyaline infiltrates around the vessel walls as has been demonstrated in patients with EPP. Homogenous PAS-positive thickening could be demonstrated in the vessel walls of the upper dermis in the sun-exposed skin in nine symptomatic patients with VP from different series (Rimington et al. 1967; Epstein et al. 1973; Corey et al. 1980, Maynard and Peters 1992; Grabczynska et al. 1996). Blisters have subepidermal localization like those in PCT.

A direct immunofluorescence study (DIF) performed in six patients with VP has revealed immunoglobulin G (IgG) and/or immunoglobulin M (IgM) depositions in the vessel walls of the upper dermis in the sun-exposed skin (Epstein et al. 1973; Corey et al. 1980; Maynard and Peters 1992; Grabczynska et al. 1996). In two of them faint complement and fibrinogen deposits have been demonstrated in the vessel walls and IgG fluorescence has been present in the epidermal basement membrane (Epstein et al. 1973). EM of the skin changes has previously been done in one patient only (Epstein et al. 1973). An extensive reduplication of the basal lamina could be demonstrated in the upper dermal vessel walls of the clinically involved skin. Widened perivascular spaces contained finely fibrillar material which extended beyond the reduplicated basal laminae. The epidermal basement membrane showed a reduplication of basal lamina.

1.5. Diagnostics of VP

1.5.1. Symptomatic patients

In VP patients who experience clinical symptoms fluorometric assay of plasma porphyrin emission spectrum shows maximum at 626 nm which is a specific for VP (Figure 7) (Poh-Fitzpatrick 1980; Long et al. 1993). The sensitivity of plasma scanning in symptomatic patients varies from 85% to 100% depending on the clinical patient data (Poh-Fitzpatrick 1980; Long et al. 1993; von und zu Fraunberg and Kauppinen 2000; Hift et al. 2004a). In VP, plasma porphyrins are a mixture of porphyrins in which the main component is a covalently bound to dicarboxylic porphyrin-protein complex combined with other porphyrins such as protoporphyrin IX, harderoporphyrin,

uroporphyrin and 5-carboxyl porphyrin (Poh-Fitzpatrick 1980; Longas and Poh-Fitzpatrick 1982). Interestingly, the level of plasma porphyrins has no correlation with the presence of skin symptoms of VP (Day 1986).

Faecal excretion of porphyrins is increased in patients with symptoms with the content of protoporphyrin exceeding that of coproporphyrin (Anderson et al. 2001). Bacterial metabolism within the intestine, gastrointestinal bleeding and intake of haem-containing food may cause a nonspecific increase of faecal protoporphyrin content and give false positive results (Zaider and Bickers 1998). Urinary excretions of coproporphyrin and uroporphyrin may vary widely but coproporphyrin predominates usually (Corey et al. 1980). Excretions of porphyrins in the urine and faeces show inter- and intra-individual variation and overlap with other porphyrias. It limits the usefulness of biochemical analysis in the diagnostics of VP (von und zu Fraunberg et al. 2002). In the Finnish series including 103 VP patients, high urinary coproporphyrin level predicted clinical symptoms of VP, and normal faecal protoporphyrin level predicted freedom of these symptoms (von und zu Fraunberg et al. 2002). During an acute attack urinary excretions of ALA and PBG are increased similarly to that of an acute attack of AIP.

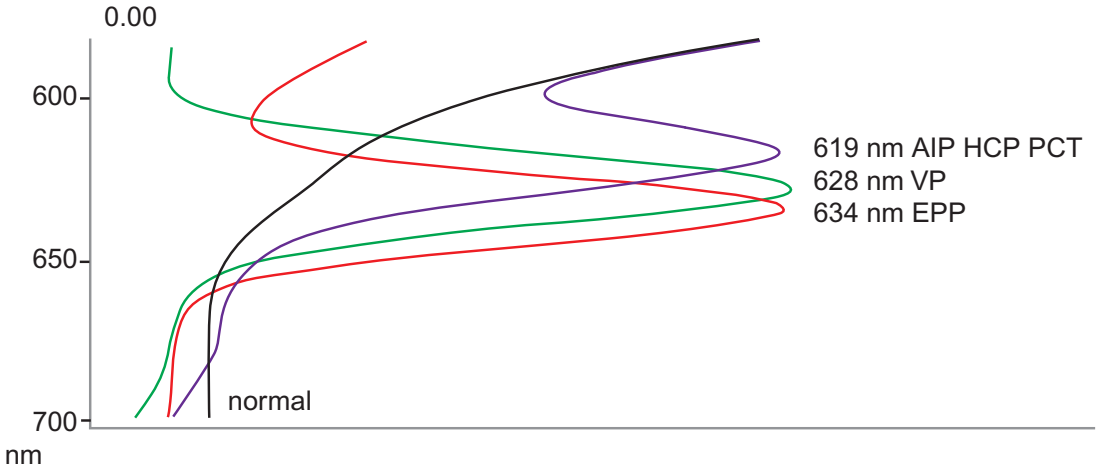


Figure 7. Fluorescence emission spectra of plasma porphyrins in different porphyrias

1.5.2. Asymptomatic patients

The porphyrin measurements fail to identify mutation carriers before puberty. The assays for plasma fluorescence and faecal porphyrins are not sensitive enough to detect all mutation carriers also in adults (Long et al. 1993; von und zu Fraunberg and Kauppinen 2000; Hift et al. 2004a). The sensitivity of plasma scanning in adult asymptomatic family members varies from 76% to 86%, but in individuals younger than 16 years the sensitivity remains at 0-25% (Long et al. 1993; Hift et al. 2004a). The assessment of the PPOX activity in lymphocytes may detect symptom-free family members, but the laborious technique limits its suitability for screening test (Deybach et al. 1981; Da Silva et al. 1995). DNA analysis provides the only accurate method to confirm the diagnosis of

VP and to detect gene carriers in the families, provided that a mutation in the PPOX gene has been identified (von und zu Fraunberg and Kauppinen 2000; Hift et al. 2004a).

1.6. Homozygous variegate porphyria

To date a total of 15 patients with homozygous VP have been identified world-wide (Kordac et al. 1984; Murphy et al. 1986; Mustajoki et al. 1987; Coakley et al. 1990; Norris et al. 1990a; D'Alessandro Gandolfo et al. 1991; Hift et al. 1993; Roberts et al. 1998; Corrigall et al. 2000; Poblete-Gutierrez et al. 2006). The mutation analysis has revealed that eight of the ten patients studied have been heteroallelic (Frank et al. 1998a; Roberts et al. 1998; Corrigall et al. 2000; Palmer et al. 2001; Poblete-Gutierrez et al. 2006) and only two patients, who are consanguineous, have been homoallelic for the PPOX gene mutations (Roberts et al. 1998). Despite the high frequency of the R59W mutation in South Africa, no R59W homozygote patient has been diagnosed suggesting that homozygosity for the R59W mutation is lethal (Corrigall et al. 2000).

In contrast to heterozygous VP, the homozygous form manifests usually as early as in infancy even within the first days of life (Hift et al. 1993). Of 15 patients, 14 subjects have experienced severe photosensitivity with blisters and erosions followed by scars on the sun-exposed areas, especially on the back of the hands and face, similarly to that in heterozygote cases (Table 4). Scarring of the head skin may lead to alopecia. In two South African cases changes in an unexposed skin including thickening of the arm skin have been described as well (Hift et al. 1993; Corrigall et al. 2000). All patients with severe photosensitivity have hand deformities ranging from clinodactyly to shortening of the fingers (Table 4).

Patients with homozygous VP experience numerous symptoms which are atypical for heterozygous condition including neurological disturbances, mental retardation and short stature (Table 4) (Hift et al. 1993; Roberts et al. 1998). Occasionally these symptoms may be present before the photosensitivity occurs (Murphy et al. 1986; Hift et al. 1993). One patient with homozygous VP has experienced only severe neurological disturbances and acute attacks (Coakley et al. 1990). The diagnosis of homozygous VP in this patient has been later queried by other investigators based on lack of skin symptoms (Hift et al. 1993). The adult onset of clinical manifestations has recently been described as exceptional cases in two South African sisters with the same heteroallelic PPOX mutations (Corrigall et al. 2000). In contrast to other homozygous cases, in whom acute attacks were absent, one of the sisters has experienced acute attacks in addition to photosensitivity, but her sister experienced only mild photosensitivity resembling heterozygous state rather than homozygous state.

Biochemical characteristics of homozygous VP include an extremely low PPOX activity (0-20% of the normal lower range) measured in lymphocytes or fibroblasts and an increased erythrocyte protoporphyrin concentration (7-10-fold compared to the upper normal range), which is predominantly zinc-chelated. In addition, plasma porphyrins emission peak appears at 626 nm and excretions of faecal and urinary porphyrins are increased variably as seen in heterozygous VP (Kordac et al. 1985; Hift et al. 1993). The urinary excretion of PBG and ALA is usually normal.

Table 4 Clinical characteristics of homozygous VP patients

Authors[1]	Kordac		Murphy		Mustajoki	Coakley	Norris	Gandolfo	Hift		Roberts		Corrigal		Poblete-Gutierrez	
Number of patients	N=2		N=2		N=1	N=1	N=1	N= 1	N=2		N=2		N=3		N=1	
Gender	M	F	M	F	M	F	F	M	F	M	M	F	F	F	F	M
Age of onset	?	?	2 y	9 mo	5 days	12 mo	18 mo	< 1 y	days	5 mo	8 mo	9 mo	10 mo	19 y	?	6 mo
Mental retardation	+	+	-	-	-	+	+	+	-	+	-	-	-	?		-
Convulsions	+	?	?	+	-	+	+	+	+	+	-	-	?	?		-
Growth retardation	+	?	+	+	-	?	-	?	+	+	-	+	-	?		+
Hand deformities	+	?	+	+	+	?	+	+	+	+	+	+	+	+		+
Nystagmus	+	+	-	-	-	?	-		+	+	-	-	+	?		-
Sensory neuropathy	?	?	?	?	+	?	-	?	+	-	?	?	+	?		-
Acute attacks	-	-	-	-	-	+	-	-	-	-	-	-	-	+		-
Death	-	-	-	-	-	+ 13 y	-	-	-	-	-	-	-	-		-
Skin symptoms	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Photosensitivity	+	+	+	+	+	-	+	+	+	+	+	+	+	+		+
Blisters, erosions Scars in the hands and face	?	?	+	+	+	?	+	+	+	+	?	?	+	?		+
Scarring alopecia	?	?	?	?	+	?	-	+	?	?		?	?	?		
Perioral furrows	?	?	+	+	+	?	+	?	?	?	?	?	+	?		+
Hypertrichosis	?	?	+	-	-	?	-	?	?	+	?	?	-	+		?
Hyperpigmentation	?	?	+	+	-	?	?	?	+	+	?	?	?	?		?
Skin changes in an unexposed skin	?	?	+	?	-	?	?	?	+	?	?	?	+	?		?

[1]Kordac et al. (1984); Murphy et al. (1986); Mustajoki et al. (1987); Coakley et al. (1990); Norris et al. (1990a); D'Alessandro Gandolfo et al.(1991); Hift et al. (1993); Roberts et al.(1998); Corrigall et al. (2000); Poblete-Gutierrez et al. (2006)

? =not applicable

1.7. Treatment of VP

Administration of haem derivatives including haemin and haem arginate is the most effective treatment of acute attacks of VP (Mustajoki et al. 1989). Haem suppresses ALAS, the rate-limiting enzyme of haem biosynthesis, by a negative feedback control and decreases the production of haem precursors in the liver (Watson et al. 1978; Mustajoki et al. 1989). The precipitating factors must be eliminated when a patient is treated during an attack and symptomatic treatment is often necessary (Kauppinen 2005).

There is no effective treatment for the skin symptoms in VP. In a small number of patients treatment using chloroquine (Fromke et al. 1978; Corey et al. 1980; Cramers and Jepsen 1980), canthaxantin (Eales 1978) or betacarotene (Corey et al. 1980) has been attempted with no or only a little clinical response. Cholestyramine, which binds porphyrins in the intestinal lumen, has been reported to be beneficial in one patient with VP (Wechsler 1975). In a recent study, which included eight patients with VP, administration of oral charcoal resulted in worsening of the skin symptoms and an increase in the levels of plasma and urine porphyrins (Hift et al. 2003). Avoidance of sun exposure and protection of the exposed skin with appropriate clothes are the only way to reduce skin symptoms to date.

Liver transplantation has been performed in two patients with VP due to non-porphyric reasons resulting in a full recovery of abnormal porphyrin metabolism (Seth et al. 2007).

1.8. Prognosis of VP

The prognosis for VP patients with acute attacks has improved over the last decades (Kauppinen and Mustajoki 1992; von und zu Fraunberg et al. 2002). Both the mortality and the frequency of severe attacks have decreased probably due to increased knowledge of inducing factors, early diagnosis with mutation screening and more specific therapy.

In the Finnish study consisting of 103 VP patients, 40% of the subjects experienced acute attacks (von und zu Fraunberg et al. 2002). Interestingly, acute attacks were present only in 14% of the VP patients diagnosed after 1980, which is significantly less than before 1980 (38%). In the series including 108 patients from France and United Kingdom, approximately 40% of symptomatic patients experienced acute attacks (Whatley et al. 1999). In a recent study from South Africa, only 4% of the 28 postpubertal individuals with the R59W mutation have had an acute attack (Hift et al. 2004b).

The prevalence of chronic renal failure and hypertension is increased among VP patients suggesting that acute porphyria increases the risk for these diseases (Kauppinen and Mustajoki 1992).To date, two cases of hepatocellular carcinoma (HCC) in VP patients have been reported (Kauppinen and Mustajoki 1988; Tidman et al. 1989) suggesting the lower incidence of HCC in VP in comparison to other hepatic porphyrias.

2. ERYTHROPOIETIC PROTOPORPHYRIA

EPP is the most common porphyria manifesting in the childhood with characteristic photosensitivity. It was clinically described for the first time in 1953 (Kosenow and Treibs 1953) and biochemically characterised in 1961 (Magnus 1961). EPP results from mutations in the gene encoding FECH which is the ultimate enzyme in the haem biosynthesis and catalyzes the insertion of ferrous iron to protoporphyrin IX to form haem (Figure 2). The reduced activity of FECH causes overproduction of protoporphyrin in erythropoietic cells with accumulation of protoporphyrin in erythrocytes and consequently in plasma, skin and liver (Todd 1994).

2.1. Prevalence of EPP

The prevalence of EPP in European countries has been estimated to be 0.5-1.3:100 000 (Todd 1994; Wiman et al. 2003a). A recent survey from United Kingdom gave a prevalence of EPP 0.7:100 000 and from Scotland 2.3:100000 respectively (Holme et al. 2006b; Dawe 2006). The first Finnish family with EPP has been reported in 1973 (Hopsu-Havu et al. 1973).

2.2. Inheritance and molecular genetics of EPP

The gene encoding FECH has been mapped to the long arm of chromosome 18 in region q21.3 (Taketani and Fujita 1995). The gene is 45 kb in size and contains 11 exons. The cDNA for FECH gene is 1269 bp in size and only one isoform of the enzyme has been demonstrated (Nakahashi et al. 1990). Since the first mutation of FECH gene was reported in 1991 (Lamoril et al. 1991) about 100 different mutations have been identified by October 2007 (Human Gene Mutation Database :http://www.hgmd.org).

Inheritance of EPP is considered to be autosomal dominant with low clinical penetrance because less than 10% of the mutation carriers develop clinical symptoms (Schneider-Yin et al. 2000). In recent studies autosomal dominant inheritance was verified in 95% of French patients with EPP and autosomal recessive inheritance in 3-4% of French and British series (Whatley et al. 2004; Gouya et al. 2006). A large pedigree analysis performed in the Netherlands before the era of molecular diagnostics has suggested that a clinically manifest disease is a result of the coinheritance of deleterious FECH allele and **"**low-expressed normal**"** FECH allele from a healthy parent (Went and Klasen 1984; Norris et al. 1990b). The different results of the lymphocyte FECH analysis in patients, their symptomatic and asymptomatic parents also suggested that EPP is not transmitted in a simple dominant fashion (Norris et al. 1990b).

The DNA studies indicate that the coinheritance of a non-mutant, FECH allele including a single nucleotide polymorphism, IVS3-48T>C, in intron 3, and a mutated FECH allele causes aberrantly spliced FECH mRNA which leads to the decreased level of normal mRNA and additional reduction of FECH activity (Gouya et al. 2002). This is consistent with the observations that the level of FECH activities measured from lymphocytes of symptomatic patients is 10-40% of that of normal, while that of asymptomatic patients is approximately 50% of the normal (Todd 1994; Schneider-Yin et al. 2000). In several series the presence of IVS3-48C polymorphism and FECH mutation has been demonstrated in patients with the clinically manifest disease and the absence of polymorphism in symptom-free patients respectively (Risheg et al. 2003; Wiman et al. 2003a; Bloomer et al. 2005; Bloomer et al. 2006). Thus, EPP acts as a recessive disease even though the deleterious FECH mutation is present in one allele. The frequency of the IVS3-48C allele in white French population is about 10% and varies in the

different ethnic populations, which may influence the prevalence of EPP in them (Gouya et al. 2006).

In addition to the IVS3-48TC polymorphism other polymorphic sites in the FECH gene have been suggested to modulate the clinical penetrance of EPP (Schneider-Yin et al. 2000). *In vitro* studies have suggested that FECH mutations affecting the certain sites of dimeric FECH protein may also cause clinically overt EPP in the absence of the modifying polymorphic allele (Najahi-Missaoui and Dailey 2005). In addition, myelodysplastic process may affect the FECH gene in haematopoietic cell line resulting in late-onset EPP without an inherited mutation (Aplin et al. 2001).

Two mouse models of EPP have been developed including a strain with a homozygous point mutation (M98K) in the FECH gene and a heterozygous strain with exon 10 deletion in the FECH gene (Tutois et al. 1991; Magness and Brenner 1999). In the first case phenotype is characterised by severe photosensitivity and grave liver dysfunction, while the other mouse model exhibits only a mild disease with no liver disease. The animal models may provide tools for studying the molecular basis and pathogenesis of human disease and for the development of a better treatment of EPP (Richard et al. 2004; Abitbol et al. 2005).

2.3. Clinical features of EPP

2.3.1. Photosensitivity

EPP is clinically manifested by acute photosensitivity that usually commences in the early infancy or the first years of life. In a recent report from the United Kingdom, the median age at the onset of the symptoms was one year (range 0-12 years) (Holme et al. 2006b). The most characteristic skin symptom of EPP is a burning pain within a few minutes to hours after a sun exposure (Table 5) (Murphy 2003). The exposure through a window glass or thin clothing may provoke symptoms. Cooling of the skin by cold water is typical relief for pain expressed by patients with EPP (Todd 1994). Although in the earlier reports itching has been reported in up to 88% of the patients, painful sensations are currently the leading initial symptom of photosensitivity (>90%) (de Leo et al. 1976; Lehmann et al. 1991; Holme et al. 2006a). This difference may be related to the interpretation of symptoms rather than to a real change of clinical characteristics. The pain and itching may be related to the mast cell activation and the release of histamine and cytokines (Lim et al. 1987; Glover et al. 1990) and down-regulation of epidermal eicosanoid metabolism as a consequence of phototoxic reaction (He and Lim 1991).

Painful sensations are followed by swelling of the exposed areas. In the recent survey this was present within six hours after solar exposure (Holme et al. 2006a). The swollen skin is usually normal in colour, but it may also be pale or erythematous. Sunburn-like reactions after short solar exposure have been reported (Meigel et al. 1981). Prolonged exposure may cause superficial vesicles followed by crusting on the facial skin. Petechiae and purpura following the swelling and bullous lesions after the overexposure to the sun are unusual skin manifestations of EPP (Torinuki and Miura 1983; Keller and Hornstein 1983; Lehmann et al. 1991; Patel et al. 2000).

Duration of exposure, which provokes skin symptoms, may vary on the subsequent days in such a way that the patient tolerates the sun exposure for several hours on the first day, but the next day skin symptoms appear just after a few minutes of exposure (Todd 1994). This event, called priming phenomenon, has been reported in 85% of patients (Holme et al. 2006a). It could be explained by a two-phased phototoxic reaction in the endothelial cells of the skin vessels (Brun et al. 1990). On the first day the skin is “primed” by light-induced transfer of

protoporphyrin from erythrocytes to the endothelial cells as demonstrated in cell cultures, and an additional exposure evokes promptly the damage in the protoporphyrin-enriched endothelial cells (Poh-Fitzpatrick 1989; Brun et al. 1990).

Chronic skin changes in EPP include linear and pitted scars on the cheeks and chin, furrows on the lips, perioral rhagades and thickening of the skin on the knuckles and the interphalangeal joints (Murphy 2003). Indurated plaques with firm papules in the shoulder region have been described in only one EPP patient (Meigel et al. 1981).

Since the painful sensations may be the only manifestation of photosensitivity in EPP or the skin signs are mild and transient, the correct diagnosis may be delayed or overlooked (Murphy 2003). In a recent study the median age at the time of the diagnosis of EPP was 12 years, but in one third of the patients the diagnosis was not confirmed until the age of 20 years or later in spite of an early onset of symptoms (Holme et al. 2006a).

The onset of skin symptoms of EPP in the adulthood is rare. Only 12 adult patients with the first clinical manifestations at the age of 33-73 years have been reported (Murphy et al. 1985; Fallon et al. 1989; Yamamoto et al. 1994; Henderson et al. 1995; Goodwin et al. 2006). Of those, eight patients had associated sideroblastic anaemia or other myelodysplastic syndromes or myeloproliferative disorders (Lim et al. 1992; Shirota et al. 2000; Aplin et al. 2001; Goodwin et al. 2006; Sarkany et al. 2006). The development of EPP in these patients is associated with an acquired somatic mutation in the FECH gene in haematopoietic cells (Aplin et al. 2001; Goodwin et al. 2006). Only one patient with EPP with first clinical manifestation at middle-age and a germ line mutation in the FECH gene has been reported (Berroeta et al. 2007).

Table 5. Skin symptoms in patients with EPP

Skin symptoms	Lehmann[1] N=20		de Leo[2] N=32		Holme[3] N=223	
I. Acute photosensitivity		%		%		%
Burning sensations	20	100	31	97	187	85
Pruritus	n.a.		28	88	n.a.	.
Swelling	13	65	30	94	179	80
Erythema	17	85	22	69	45	20
Blisters	3	15	1	3	37	17
Crusting lesions	3	15	n.a.		32	14
Petechiae or purpura	3	15	1	3	20	9
II. Chronic lesions						
Linear and pitted scars	3	15	6	19	149	67
Thickening of the skin on the knuckles	6	30	n.a.		78	35

[1]Lehmann et al. (1991); [2]de Leo et al. (1976); [3]Holme et al. (2006a) n.a. not applicable

2.3.2. Liver disease

Hepatobiliary complications have been reported in 20-30% of the EPP patients (Doss and Frank 1989; Meerman 2000). Usually the liver disease is mild and limited to increased serum transaminase level and only mild abnormalities in the liver histology (Cripps and Goldfarb 1978; Meerman 2000). Gallstones, which develop as a result of accumulation of protoporphyrin in bile and may occur as early as in childhood, have been reported in 8-10% of the cases (de Leo et al. 1976; Doss and Frank 1989; Holme et al. 2006a).

Less than 5% of the EPP patients develop a rapidly progressing cholestasis leading to a fatal

liver failure which is the most severe sequel of EPP occurring even in childhood (Todd 1994; Meerman 2000). An extensively high or progressively increasing erythrocyte protoporphyrin level and increased urinary coproporphyrin excretion, predominantly isomer I, may precede liver dysfunction (Doss and Frank 1989). Cholestatic injury and dysregulation of mitochondrial respiratory chain enzymes leading to oxidative stress have been proposed as etiologic factors of liver failure (Meerman 2000; Bloomer et al. 2005). Although liver complications have been linked to compound heterozygosity of the defective FECH mutation (Cox et al. 1998), and to exon deletion and a C175 stop codon (Schneider-Yin et al. 1994) and "null allele" mutations in FECH gene (Minder et al. 2002), it has not been possible so far to assess the genetic risk for development of liver failure. Mouse model studies suggest that genetic background modulates at least the severity of liver injury in EPP (Abitbol et al. 2005).

The histopathological examination of the liver may reveal protoporphyrin deposits presenting as pigment with a characteristic birefringence on polarizing microscopy and needle-like crystals in hepatocytes and bile canaliculi (MacDonald et al. 1981; Komatsu et al. 2000). In EM crystal-containing vacuoles in hepatocytes and bile canaliculi have been demonstrated in EPP patients without apparent clinical liver disease and with normal histopathology (MacDonald et al. 1981; Rademakers et al. 1990). Moreover, portal or periportal fibrosis and mononuclear inflammatory cell infiltrates may be present without any clinical evidence of liver dysfunction (Cripps and Goldfarb 1978; MacDonald et al. 1981). In advanced liver disease micronodular cirrhosis and cholestasis are present in addition to a massive accumulation of protoporphyrin (Cripps and Goldfarb 1978; MacDonald et al. 1981).

2.3.3. Haematological changes

Mild anaemia including microcytosis and hypochromia has been reported in 20-60% of EPP patients (de Leo et al. 1976; Baart de la Faille et al. 1991; Holme et al. 2007). A downward shift in haemoglobin level has been a constant finding also in those patients without anaemia (Holme et al. 2007). Serum ferritin level was decreased in the majority of the patients (2/3), but serum soluble transferin receptor-1 and iron concentrations were normal suggesting that the bone marrow has been adopted to impaired erythropoiesis, and absorption and supply of iron is inhibited to avoid storage of toxic iron compounds (Holme et al. 2007). Studies in the FECH mouse model demonstrated that microcytic anaemia associated with FECH deficiency was not caused by iron deficiency but was accompanied by a redistribution of iron from the liver to the spleen (Lyoumi et al. 2007). Iron deficiency in EPP patients may also be related to mutations of iron regulatory protein 2 (Cooperman et al. 2005). There is some evidence that haem-regulated inhibitor kinase, which controls balanced globin-haem synthesis, is responsible for the hypochromia and microscytosis in EPP related anaemia as well as in iron deficiency (Chen 2007).

2.4. Histopathology of the skin in EPP

The major histopathological findings of the skin were identified soon after EPP was characterised as a separate entity. Thickening of the vessel walls of the upper dermis and amorphous deposits in and around them have been demonstrated in the sun-exposed skin (Peterka et al. 1965; Ryan 1966; Epstein et al. 1973; Baart de la Faille 1975). Similar changes were discovered in the clinically normal skin of eight patients with EPP on the dorsum of the hand (Rimington et al. 1967). In these studies no abnormalities have been demonstrated in the covered skin. Only mild epidermal changes including hyperkeratosis and acanthosis were

demonstrated (Rimington et al. 1967).

The amorphous material in and around the vessel walls is strongly PAS-positive suggesting the presence of glycoprotein (Rimington et al. 1967). In addition lipids and tryptophan have been demonstrated in the vessel walls and in the amorphous depositions (Rimington et al. 1967). In the immunohistochemical studies, collagen IV and laminin, which are glycoprotein components of normal basement membrane matrix, have been identified in the thickened vascular walls (Wick et al. 1979)**.** Connective tissue and elastic fibres constituents such as serum amyloid P component, fibronectin and vitronectin have been discovered in the vessel walls (Breathnach et al. 1983; Dahlbäck et al. 1988).

In DIF including samples of 15 patients immunoglobulins, mainly IgG, were demonstrated in the vessel walls (Epstein et al. 1973: Baart de la Faille 1975). EM revealed a concentric reduplication of the basal lamina and fine granular material at the basement membrane zone (Ryan and Madill 1968; Anton-Lambrecht and Bersch 1971; Epstein et al. 1973; Baart de la Faille 1975).

2.5. Diagnostics of EPP

2.5.1. Symptomatic patients

The biochemical diagnosis of EPP is based on the demonstration of elevated level of protoporphyrin concentration in erythrocytes (Todd 1994). The fluorescence microscopy of a blood smear from patients with EPP have demonstrated that up to 30% of the erythrocytes express bright, transient fluorescence (fluorocytes) (Zaider and Bickers 1998). Spectrofluorometric scanning of plasma shows a maximum emission at 634 nm for protoporphyrin (Figure 7) (Poh-Fitzpatrick and Lamola 1976). The excretion of protoporphyrin to faeces may be increased or normal (Todd 1994). Urinary excretion of porphyrins is usually normal, but the excretion of coproporphyrin with predominance of isomer I is increased in patients with the liver disease (Doss and Frank 1989)**.**

Increased protoporphyrin concentrations in erythrocytes occur also in iron deficiency, sideroblastic anaemia, lead poisoning and in homozygous forms of porphyria, but in these conditions protoporphyrin is mostly zinc-chelated and unable to diffuse across the cell membrane and bind to plasma proteins (Zaider and Bickers 1998) resulting in negative plasma scanning.

2.5.2. Asymptomatic patients

In the asymptomatic individuals who have inherited a FECH mutation the erythrocyte protoporphyrin concentration is commonly normal (Goerz et al. 1996). Red fluorescence of peripheral red cells may also be demonstrated in asymptomatic patients (Went and Klasen 1984).

The erythrocyte and leucocyte FECH activity is usually decreased, approximately 50% of normal, but the levels of the enzyme activity may overlap with health controls (Norris et al. 1990b; Gouya et al. 1999). As in other porphyrias, DNA analysis is the most reliable way to detect asymptomatic individuals in the families in which the mutation has been identified.

2.6. Treatment of EPP

To date no curative, standard treatment exists for photosensitivity in EPP. The oral administration of beta-carotene has been reported to increase tolerance to sunlight in 70-80% of EPP patients, and it is the most widespread treatment in EPP (Mathews-Roth et al. 1977; Poh-Fitzpatrick 1982; Lehmann et al. 1991). The photoprotective effect of beta-carotene in EPP is probably due to its ability to quench singlet oxygen or to scavenge reactive free radicals generated in phototoxic reaction (Poh-Fitzpatrick 1982). In a single placebo-controlled crossover trial with EPP patients, the efficacy of beta-carotene was poor (Corbett et al. 1977). For the clinical use the dosage of beta-carotene should be 120-180 mg/day for adults and 30-90 mg/day for children (Todd 1994). If serum carotene level is monitored, it should be maintained at 600-800 ug/dl during the treatment.

Oral vitamin C because of its antioxidant properties, may improve the tolerance to sunlight in EPP (Boffa et al. 1996). Phototherapy using PUVA (Ros 1988) or narrow-band UVB (Warren and George 1998) has alleviated photosensitivity in single cases. Administration of cysteine has been found to increase the symptom-free period during light exposure (Mathews-Roth and Rosner 2002)**.** Pyridoxine with high doses has reliefed the photosensitivity in two child patients with EPP (Ross and Moss 1990). Terfenadine inhibits partly the immediate flare reaction to light irradiation in patients with EPP (Farr et al. 1990), but its efficacy has not been proved in clinical trials. Avoidance of light exposure, use of protective clothing and application of topical UVA blocking sunscreen agents containing titanium or zinc oxide may be helpful (Murphy 2003)

Cholestyramine and ursodeoxycholic acid have been used to control the progression of liver disease (Murphy 2003). These compounds may increase hepatic excretion of protoporphyrin into the bile and interrupt the enterohepatic circulation of protoporphyrin. The efficacy of these types of treatment has not been established in controlled trials. The best management for advanced liver disease of EPP is currently liver transplantation although recurrent protoporphyrin-induced damage in the allograft has been reported in 65% of transplanted cases (McGuire et al. 2005).

In animal models the bone marrow transplantation corrects the FECH deficiency in the bone marrow resulting in improvement of photosensitivity (Pawliuk et al. 1999; Fontanellas et al. 2001). Similar results have been obtained in a patient with EPP and myelogenous leukaemia after bone marrow transplantation (Poh-Fitzpatrick et al. 2002) and in another patient with EPP who developed severe cholestasis (Wahlin et al. 2007). In the latter case the bone marrow transplantation was also curative for cholestasis. The bone marrow transplantation may provide new treatment option of EPP in the future in combination with liver transplantation which might prevent the recurrence of EPP liver disease. Haematopoietic stem cell gene therapy has corrected a progressive rise of erythrocyte protoporphyrin level and photosensitivity in mice with EPP (Richard et al. 2004).

3. PORPHYRIA CUTANEA TARDA

PCT was first described in 1937 in patients with porphyrinuria and skin symptoms in the sun-exposed areas manifesting in adulthood (Waldenström 1937). PCT results from the decreased activity of UROD in the liver accompanied by overproduction of uroporphyrin and other highly carboxylated porphyrins. In the majority of cases the disease is not related to the mutation in the UROD gene, but other individual genetic factors probably control susceptibility to the environmental factors which precipitate the clinical expression of the disease (Badminton and Elder 2005). Clinically PCT is manifested by photosensitivity of a chronic nature and by the liver disease.

3.1. Prevalence of PCT

PCT is the most common type of porphyria in Europe and USA, where the prevalence of PCT has been estimated at 4-10:100 000 (Elder et al. 1990; Anderson et al. 2001; Rossmann-Ringdahl and Olsson 2005). The highest frequency of PCT, 1:1000 (100:100 000) among the adult population, has been estimated in former Czechoslovakia and Mongolia based on the porphyrin screening of urine (Martasek et al. 1987). A high prevalence among South African Bantu people has been linked to use of alcohol and cooking in ironware resulting in hepatic siderosis (Elder 1998).

3.2. Inheritance and molecular genetics of PCT

PCT has been classified into four subgroups (Table 6) according to the heredity and the extent of the UROD defect in the tissues (Elder 1998; Anderson et al. 2001). In all of them the UROD activity is decreased in the liver (Elder 1998). In type I, called sporadic or acquired PCT, no mutations of UROD locus have been identified (Garey et al. 1993) (Human Gene Mutation Database:http://www.hgmd.org), and the UROD activity is normal when measured from patients' erythrocytes and fibroblasts (Elder et al. 1978). Familial PCT includes types II and III and has an autosomal dominant inheritance. Type II PCT is related to a mutation at the UROD locus and the activity of erythrocyte UROD is reduced to 50% of the normal range (Elder 1998). In type III PCT, which is the other form of familial PCT, the patient has the family history of PCT but the erythrocyte UROD activity is normal (Elder 1998). The fourth type of PCT is toxic caused by environmental exposure to polyhalogenated aromatic hydrocarbons which are able to inhibit UROD leading to accumulation of uroporphyrin (Moore et al. 1987). The most serious instance of toxic PCT was an outbreak of PCT in Turkey 50 years ago followed by treatment of wheat seeds with a hexachlorobenzene containing fungicide (Cripps et al. 1984). The hexachlorobenzene induced PCT in mouse has been utilised as an experimental model for human type I PCT (Smith and Francis 1983).

Based on the enzyme analysis 50-80% of patients with PCT has been classified to have type I with normal UROD activity measured in patients' erythrocytes (Doss et al. 1991; Koszo et al. 1992; Elder 1998; Cruz-Rojo et al. 2002; Tavazzi et al. 2002). In Spanish series of 118 patients studied by enzyme analysis the proportions of types I, II and III have been 47%, 37% and 16% respectively (Cruz-Rojo et al. 2002). In contrast, mutation analysis of the UROD gene in 61 Spanish patients' samples revealed the corresponding proportions 66%, 26% and 8% (Mendez et al. 2007). A mutation in the UROD gene could be identified in 25% of the Danish patients with PCT indicating that the familial type is more common than previously presumed (11%)

(Bygum et al. 2003). Respectively, in Chile the frequency of type II PCT was approximately 50% based on mutation analysis (Poblete-Gutierrez et al. 2004).

Table 6. Classification of UROD related disorders[1]

Disease	Mode of inheritance	UROD activity		Mutations[2]
		Liver	Erythrocytes	
PCT				63
Type I	Sporadic, other genes involved	Decreased	Normal	
Type II	Autosomal dominant	Decreased	Decreased	
Type III	Autosomal dominant	Decreased	Normal	
Toxic PCT	Acquired	Probably decreased[1]		
HEP	Homozygous or compound heterozygous	Decreased	Decreased	10

[1]Anderson et al. (2001); [2]http://www.hgmd.cf.ac.uk/ac/all.php

Hepatoerythropoietic porphyria (HEP) is an uncommon variant of PCT caused by homozygous or compound heterozygous defects in the UROD gene leading to a severe deficiency of the UROD activity. To date less than 40 patients with HEP have been reported (Phillips et al. 2007a). HEP is manifested in the early childhood and characterised by severe photosensitivity and red-coloured urine resembling congenital erythropoietic porphyria (Phillips et al. 2007a).

Human UROD is a 42 kD polypeptide encoded by a single gene which contains 10 exons spread over 3 kb of DNA. The UROD gene has been mapped to chromosome 1p34 (Human Gene Mutation Database:http://www.hgmd.org). By October 2007 a total of 73 mutations including 10 mutations in the phenotype of HEP have been identified in the UROD gene (Human Gene Mutation Database:http://www.hgmd.org). As in other porphyrias, UROD mutations are usually family-specific. A mouse with the heterozygous null-mutation at the UROD locus has been developed for a model of familial PCT and used for studies of the mechanism of inhibition of UROD in the liver (Phillips et al. 2001; Phillips et al. 2007b).

3.3. Risk factors and associated conditions in PCT

3.3.1. Mechanism of UROD inactivation

UROD inactivation is usually induced by a combination of various factors such as exogenic compounds, mutations in the genes associated with liver metabolism or chronic viral infections (Table 7). The existence of UROD-inhibitor produced by hepatocytes has been suggested, since the protein level of UROD remains unchanged even if the catalytic activity decreases (Elder 1998). Based on more recent experimental studies in animal models and liver cell cultures, hepatic UROD can be inhibited by uroporphomethene which originates from uroporphyrinogen by iron-dependent oxidation (Phillips et al. 2007b). In addition, an induction of CYP1A2 may accelerate the inhibition of UROD (Smith and De Matteis 1990; Elder 1998). Certain polymorphisms in the CYP1A2 and CYP P4501A1 genes may be associated with the susceptibility to type I and II PCT (Christiansen et al. 2000; Gardlo et al. 2003).

3.3.2. Alcohol intake

Alcohol is the major risk factor but not a prerequisite for development of PCT. According to different series in 30–90% of the cases the daily consumption of alcohol exceeds 40 g, but a dose-response relationship between alcohol intake and manifestations of PCT has not been confirmed (Elder 1999). On the contrary, PCT is a rare disease among alcoholics and additional predisposing factors are required. Alcohol most likely contributes to the inhibition of hepatic UROD in susceptible individuals by oxidative and CYP-mediated processes (Elder 1999).

Table 7. Risk factors of PCT

Predisposing factor	Frequency among PCT patients based on different patient series
Genetic	
Mutations in the UROD gene	20-50%
Mutations in the *HFE* gene	60-70%
Certain CYP1A2 polymorphisms	70%
Exposures	
Alcohol	30-90%
Oestrogen	55-70% of females
Iron overload	60-90%
Chronic viral infections	
Hepatitis B	20-70%
Hepatitis C	20-90%
HIV	15-25%
Other	
Ascorbic acid deficiency	60%[1]
Smoking	90%[2]
Haemodialysis	1-18%

[1]Sinclair et al (1997); [2]Egger et al (2002)

3.3.3. Oestrogens

An intake of oestrogen preparations for contraception and a hormone replacement has been identified as a risk factor in 55-70% of female patients with PCT (Egger et al. 2002; Rossmann-Ringdahl and Olsson 2005). Oestrogen intake has been the solitary risk factor in more than 25% of female patients (Bulaj et al. 2000) especially among those with the familial type of PCT (Sixel-Dietrich and Doss 1985). Men treated with oestrogen preparations for prostate cancer have rarely developed PCT (Sixel-Dietrich and Doss 1985). The mechanism by which the intake of oestrogens induces UROD deficiency is unclear (Bulaj et al. 2000).

3.3.4. Iron overload

Iron has probably an essential role in the inhibition of the hepatic UROD (Phillips et al 2007b). The importance of iron for pathogenesis of PCT is supported by the fact that diminishing iron stores by venesections is curative for PCT (Elder 1990; Sarkany 2001). The majority of the patients with PCT have a direct or indirect evidence of iron load in the liver. Increased serum iron and ferritin levels have been reported in 60-90% of PCT patients (Grossman et al. 1979;

Rocchi et al. 1986; Fargion et al. 1996; Bulaj et al. 2000). In liver biopsy hepatic siderosis has been demonstrated up to 95-100% of PCT patients (Rocchi et al. 1991; Siersema et al. 1995; Bulaj et al. 2000). *HFE* mutations, which are associated with clinical hemochromatosis, are probably most commonly responsible for iron overload in PCT (Bulaj et al. 2000). At least one *HFE* mutation has been identified in 60-70% of patients with PCT in Europe and USA (Bulaj et al. 2000; Egger et al. 2002; Harper et al. 2004; Kratka et al. 2008)

The frequency of the C282Y mutation among the patients with PCT varies from 20% to 60% and that of the H63D mutation up to 50%, respectively (Sampietro et al. 1998; Bulaj et al. 2000; Cruz-Rojo et al. 2002; Egger et al. 2002; Kratka et al. 2008). The homozygous state of the C282Y mutation or the compound heterozygous state of the C282Y and H63D mutations in patients with PCT have been demonstrated in several series, and these genotypes associate particularly with abnormal iron status (Bulaj et al. 2000; Harper et al. 2004). The homozygosity for the C282Y mutation has been shown to accelerate clinical manifestations in the familiar type II of PCT (Brady et al. 2000). The S65C mutation in the *HFE* gene has no significant role in the pathogenesis of PCT (Lamoril et al. 2002; Harper et al. 2004). To date, no mutation in the transferring receptor 2 (TFR2) gene has been detected among patients with PCT (Kratka et al. 2008). Independently from the *HFE* mutations, hepatic siderosis in PCT may associate with polymorphism in the transferrin receptor-1 gene (Lamoril et al. 2002) and down-regulation of hepcidin in hepatocytes (Ajioka et al. 2008). In addition, iron overload can be induced by oral or intravenous iron preparations and by repeated transfusions especially in those having genetic vulnerability (Shehan and Huerter 2001).

3.3.5. Viral hepatitis

High frequencies of hepatitis B virus (HBV) and C virus (HCV) infections in PCT patients have been reported in several studies. A serologic evidence of HBV infection has been found in 20 - 70% of the PCT patients; the highest frequency has been reported from southern Europe (Rocchi et al. 1986; Stransky et al. 2000). Based on the published data, the frequency of HCV infection in patients with PCT has been estimated at 50%, but the frequency varies significantly depending on the country and the type of PCT (Gisbert et al. 2003). The highest frequencies of HCV infection have been reported from southern Europe (66-91%), USA (56-76%) and Japan (85%), while in northern Europe and Scandinavian countries the frequencies of HCV infection have been below 30% (Gisbert et al. 2003; Rossmann-Ringdahl and Olsson 2005). Calculated from the published data the prevalence of HCV infection in patients with type I PCT was 2-fold compared to that reported from patients with type II PCT (57% vs. 26%) (Gisbert et al. 2003). In Sweden the prevalence of HCV infection among patients with type I PCT was 36% but only 4% among those with type II PCT (Linde et al. 2005)

3.3.6. HIV infection

Around 100 cases of PCT have been reported among patients with human immunodeficiency virus (HIV) infection (Mansourati et al. 1999). In a small American series 25% of 24 patients with PCT tested for HIV was positive (Egger et al. 2002). Additional risk factors for PCT such as HBV, HCV and alcohol use were present approximately in half of HIV patients with PCT. This suggests that HIV infection may have an independent causative effect on the development of PCT (Mansourati et al. 1999). The proposed mechanisms which could lead to an alteration in porphyrin metabolism during HIV infection include impairment of the hepatic cytochrome

oxidase system by HIV infection or by associated opportunistic infections, alteration of hepatic oestrogen metabolism and increase of hepatic iron store as a result in ineffective haematopoiesis caused by HIV infection (Mansourati et al. 1999; Almehmi et al. 2005).

3.3.7. Haemodialysis

Haemodialysis for chronic renal failure may precipitate PCT. The frequency of PCT varies from 1 to 18% of the patients receiving haemodialysis (Shieh et al. 2000). The development of PCT in haemodialysed patients may be related to latent PCT or to the deficient UROD activity caused by azothemia and accumulation of porphyrins because of impaired excretion and poor dialysis (Shieh et al. 2000). Aluminium intoxication from the equipment used for dialysis may also disturb haem synthesis (Shieh et al. 2000).

3.4. Skin Symptoms of PCT

3.4.1. Clinical manifestations of skin symptoms

Skin symptoms in PCT of different subtypes are similar. The onset is usually after the fourth decade of life, but in type II disease can manifest earlier than in type I, sometimes even in childhood (Elder 1998). The characteristic skin signs include blistering and fragility of the sun-exposed skin resulting in raw erosions after minor trauma (Table 8) (Grossman et al 1979, Poh-Fitzpatrick 1982). Occasionally the blisters may develop to large bullae. The eroded skin lesions heal slowly with crusting, scarring and milia formation like in VP. The skin lesions occur regularly on the dorsal sides of the hands and fingers, but also on the other chronically sun-exposed areas as the face, neck, arms, legs and feet. The skin symptoms arise in summer and continue in autumn beyond the sunny season.

The chronic skin signs of PCT comprise hypertrichosis, hyperpigmentation, solar elastosis and scleroderma-like changes (Table 8). Hypertrichosis on the face skin is a frequent finding reported in two-thirds of PCT patients (Grossman et al. 1979; Boffa et al. 1995). Usually it is located in the upper cheek and the periorbital areas. Hyperpigmentation on the face skin without preceding bullae or erosions in the same region has been identified in half of patients (Grossman et al. 1979). It is usually mottled in appearance, but more diffuse and reticulated pattern has also been described (Grossman et al. 1979; Sarkany 2001). Hypertrichosis and/or hyperpigmentation may be the only clinical signs of PCT (Fritsch et al. 1998). In 1.6-18% of PCT patients scleroderma-like lesions occur both in the light exposed and covered skin, most commonly on the head and upper chest (Grossman et al. 1979; Friedman and Doyle 1985). In single cases the localised scleroderma has been the only cutaneous manifestation concomitant with the increased uroporphyrin excretion in the urine (Friedman and Doyle 1985). Scleroderma-like changes preceed typical signs of PCT or develop during years after the onset of typical skin symptoms of PCT in untreated cases (Friedman and Doyle 1985).

Figure 8. Typical blisters and bullae on the dorsal sides of the hands in a patient with PCT (on the courtesy of the patient)

The skin symptoms of PCT are indistinguishable from those in VP and HCP. Moreover, in pseudoporphyria induced by various drugs and long-term haemodialysis identical to those of PCT skin symptoms occur but no biochemical porphyrin abnormalities can be found (Green and Manders 2001). PCT has been reported to be associated with systemic lupus erythematosus (Cram et al 1973) although no definite relationship has been found (Griso et al 1989). Photodamage of the conjunctiva has been suspected in a few patients with PCT related to high concentration of uroporphyrins (Hammer and Korom 1992).

Table 8. Skin symptoms in PCT
Skin fragility of the sun-exposed skin
Erosions and blistering in the the sun-exposed skin
Scarring and hypopigmentation on the old skin lesions
Milia formation on the old skin lesions
Hypertrichosis on the face skin
Hyperpigmentation on the face skin
Scleroderma like lesions, also on the sun-protected skin
Photo-onycholysis

3.5. Histopathology of the skin in PCT

LM of the blister in PCT reveals that it has subepidermal localisation with scanty inflammatory cells (Kemmer et al. 1988; Maynard and Peters 1992). Collagen IV and laminin have been demonstrated at the base of the blister by immunohistochemical studies suggesting that the blister develops at the level of lamina lucida above lamina densa (Pardo and Penneys 1990; Dabski and Beutner 1991). In EM the level of cleavage has been identified both above the basal lamina and in the dermal site (Kint and De Weert 1978; Klein et al. 1983). The initial stage of the split formation has been hypothesised to arise above the basal lamina, and then additional factors such as the light energy and mechanical trauma aggravate the injury to more profound dermolytic blister (Klein et al. 1983; Nagato et al. 1987). A characteristic morphologic feature of PCT blister is that dermal papillae beneath the blister retain their original configuration resulting in undulating appearance of the blister floor (Maynard and Peters 1992). In addition, caterpillar bodies, which are eosinophilic bodies containing degenerating keratinocytes and basement membrane material have been demonstrated in the epidermis overlying the blister (Egbert et al. 1993; Raso et al. 1996). They are considered highly specific for PCT but have been found only in around 40% of cases (Fung et al. 2003).

In the vessel walls of the sun exposed skin homogenous thickening containing PAS positive material has been identified (Epstein et al. 1973; Kemmer et al. 1988). EM has demonstrated that thickening of the vessel walls is caused by reduplication of basal lamina of the basement membrane zone and excess of fine fibrillar and an amorphous material around the vessels (Epstein et al. 1973; Kemmer et al. 1988). No microscopic changes have been observed in the vessel walls in the covered skin (Rimington et al. 1967; Epstein et al. 1973).

DIF reveals depositions of IgG and variably C3 and fibrin in the vessel walls of the upper dermis in the skin lesion (Epstein et al. 1973; Kemmer et al. 1988). Deposits of immunoglobulins and complement have been considered to originate from circulation rather than from the activation of complement system (Epstein et al. 1973). Deposits of complement 5b-9 in the vessel walls has been demonstrated in skin samples of patients with PCT similar to those with diabetes mellitus, in which vascular changes of the skin are comparable to PCT (Vasil and Magro 2007). The authors propose that complement is activated by irradiated uroporphyrins resulting in complement 5b-9 deposits in the vessel walls. Thus, similar complement activation and histological changes may also occur by glycolysation as in diabetes. In the epidermal basement membrane zone IgG and C3 have been variably found (Epstein et al. 1973).

The deposits of the vessel walls except immunoglobulins have been characterised incompletely in PCT. Collagen IV has been demonstrated in the vessel walls in a single case (Krajnc et al. 1998). Vitronectin, which is a cell-adhesive glycoprotein in plasma and dermal elastic fibres, has been demonstrated in the perivascular deposits in PCT probably because it can attach abnormal protein deposits (Dahlbäck et al. 1988). The presence of vitronectin has been hypothesised to be associated with the late-stage inhibition of the activated complement system in the phototoxic reaction (Hintner et al. 1991).

3.6. Liver disease in PCT

3.6.1. Hepatopathy

In addition to skin symptoms the liver disease is another major clinical manifestation of PCT and occurs in 80-90% of the cases (Sarkany 2001). Alcohol ingestion, viral hepatitis and iron overload are often present concomitantly contributing to the development of the liver disease. Serum transaminase levels are increased in 50-80% of patients (Grossman et al 1979; Moore et al. 1987). Abnormalities in the liver function tests occur also in those patients with PCT who have no overt liver disease or known risk factors suggesting that excess of uroporphyrin itself may cause hepatopathy (Campo et al. 1990). The liver disease in untreated PCT can progress to cirrhosis which has been reported in 20-30% of the patients (Grossman et al 1979; Cortes et al 1980). Antibodies to human liver cytosolic antigens are present frequently in type I PCT and they are associated with HCV infection and the severity of liver disease (Ma et al. 2001).

3.6.2. Liver histology

Increased iron deposits are nearly always present in liver biopsy specimens of patients with PCT (Campo et al. 1990). Different non-specific morphological changes including fatty degeneration, focal lobular necrosis, portal inflammation and fibrosis have been demonstrated in up to 90% of the patients and they can vary from negligible to severe (Campo et al. 1990). Needle-shaped uroporphyrin crystals can be demonstrated in cytoplasm of hepatocytes (Cortes et al. 1980; Fakan et al. 1998). In EM uroporphyrin crystals are located close to ferritin-like iron deposits indicating the crucial role of iron in pathogenesis of PCT (Siersema et al. 1995). The simultaneous presence of haemosiderin in periportal hepatocytes, fatty changes and portal inflammation has been demonstrated to be more common in PCT than in other liver diseases. In the latter group haemosiderin is more often present in Kupfer cells (Campo et al. 1990)

3.6.3. Hepatocellular carcinoma

Patients with PCT have a high prevalence of hepatocellular carcinoma (HCC). The highest frequency of HCC, 47% of the patients with PCT, was reported from the autopsy series done in former Czechoslovakia (Kordac 1972). HCC has been identified in 13-24% of PCT patients in various small series (Solis et al. 1982; Siersema et al. 1992; Linet et al. 1999). Patients with PCT and chronic hepatopathy who have been treated with phlebotomies to remission still have a 4-fold risk for HCC in comparison to control patients with chronic liver disease but no PCT (Fracanzani et al. 2001). In a recent survey from Spain HCC was detected only in one of 39 patients (2.6%) during the 10-year follow-up (Gisbert et al. 2004). Thus, the incidence of HCC was less than 1% per patient-year of the follow-up. A long symptomatic period before the treatment, the presence of cirrhosis and the chronic HCV infection are the major risk factors for HCC in PCT (Siersema et al. 1992).

3.7. Diagnostics of PCT

The biochemical hallmark of PCT is overproduction of uroporphyrin in the liver which is released into circulation and excreted into the urine. Urine contains uroporphyrin, predominantly isomer I, and 7-carboxyl porphyrin, and lesser amount of 6- and 5-carbpoxyl porphyrins (Moore et al. 1987).

Isocoproporphyrin is a typical finding in faeces in PCT, because in the lack of UROD the propionic side chains of 5-carboxylate intermediate are decarboxylated and oxidised to a vinyl group probably by coproporphyrinogen oxidase resulting in dehydroisocoproporphyrinogen. The latter is converted to isocoproporphyrin probably by bacterial degradation in the bowel and can be identified (Moore et al. 1987; Cooper et al. 2005).

Plasma porphyrin content is a mixture of porphyrins, uroporphyrin being the main component. The spectrofluorometric assay of plasma porphyrins detects fluorescence maximum at 619 nm (Figure 7) (Poh-Fitzpatrick and Lamola 1976). The elevation of urinary uroporphyrin and 7-carboxyl porphyrin level and the demonstration of typical plasma fluorescence in an individual with typical skin lesions confirm the diagnosis of PCT. Measurement of UROD activity is used to distinguish type II PCT (Elder et al. 1989).

3.8. Treatment in PCT

Elimination of exogenous precipitating factors such as alcohol, oestrogen compounds and iron supplements improves the effect of active therapy and prolongs the duration of remission after treatment (Sarkany 2001). In some cases, cessation of alcohol intake and predisposing drugs can result, although slowly, in clinical and biochemical remission without other treatment action. In the majority of patients with PCT**,** active treatment such as phlebotomy or low dose chloroquine are required and has been used successfully for years.

Phlebotomy corrects the overproduction of porphyrins probably via reduction of the hepatic iron stores (Grossman and Poh-Fitzpatrick 1986). Phlebotomies are performed at weekly or biweekly intervals (400-500 ml/phlebotomy) until the haemoglobin concentration falls to 100-110 g/l or serum ferritin level is below the normal reference value (Sarkany 2001). Four to 10 litre of blood must usually be removed for the therapeutic effect. The improvement of clinical symptoms after starting of phlebotomies occurs usually within 6 months, and excretion of porphyrins normalises within a year in general. In haemodialysis-associated PCT small volume phlebotomies in combination with the high-dose erythropoietin administration have been effective (Shieh et al. 2000).

A dose of 125-250 mg of chloroquine twice a week usually produces clinical improvement during four months and biochemical remission (urinary uroporphyrin excretion below 100 nmol/d) within a year (Sarkany 2001). The effect of low-dose chloroquine is probably related to the formation of porphyrin complexes in the liver cells (Scholnick et al 1973), and the compounds are excreted massively at the beginning of the treatment via bile and urine (Rossmann-Ringdahl and Olsson 2007). Simultaneously transient increase of the serum aminotransferases levels may be observed.

AIMS OF THE PRESENT STUDY

1. To study occurrence of cutaneous porhyrias in Finland
2. To study etiological factors including genetical changes associated with cutaneous porphyrias
3. To study clinical manifestations of VP, EPP and PCT and their response to treatment
4. To investigate the histopathological changes and pathogenesis of the skin lesions in VP, EPP and PCT

In the beginning of this study no detailed information about Finnish patients with cutaneous porphyrias was available except 55 patients with VP studied by Professor Pertti Mustajoki (Mustajoki 1980) and one Finnish family with EPP reported by Professor Väinö Hopsu-Havu (Hopsu-Havu et al. 1973).

MATERIAL AND METHODS

1. PATIENTS

1.1. Variegate porphyria

Of total of 156 Finnish VP patients (von und zu Fraunberg et al. 2002) belonging to 22 different families, 20 patients were selected for the histolopathological studies. One of the patients with VP was homozygous for the deficient PPOX (Mustajoki et al. 1987). All patients with VP were diagnosed at the University Central Hospital of Helsinki during the period 1966-2006.

The diagnosis of VP in symptomatic patients was based on the fragility of the sun-exposed skin and/or acute neurovisceral attacks and on increased protoporphyrin excretion exceeding coproporphyrin excretion in the faeces (Li et al. 1986). Chracateristic plasma porphyrin fluorescence emission spectrum (maximum at 626 nm) confirmed the diagnosis of VP (Poh-Fitzpatrick 1980). Decreased lymphocyte PPOX activity (Deybach et al. 1981) was observed in at least one of the family members.

In symptom-free patients the diagnosis was based on the family history of VP and on increased faecal protoporphyrin excretion and/or a decreased lymphocyte PPOX activity or on mutation analyses (von und zu Fraunberg et al. 2002). The diagnosis of the homozygous patient was based on the extremely low (10%) lymphocyte PPOX activity in the proband and on half normal enzyme activity in his parents who were cousins (Mustajoki et al. 1987). The information on the clinical symptoms was obtained by clinical examination, by telephone interview and/or using data from earlier studies (Mustajoki and Koskelo 1976; Mustajoki 1978; Mustajoki 1980).

1.2. Erythropoietic protoporphyria

Of 48 Finnish patients with EPP belonging to 15 different families, eight patients were studied using histopathological methods. Finnish EPP patients were diagnosed at the University Central Hospitals in Helsinki, Turku, Tampere and Oulu and in the Central Hospitals in Joensuu, Vaasa and Pori since the 1970's.

The diagnosis of EPP in symptomatic patients was based on acute photoreactions characterised by painful swelling and erythema of the sun-exposed skin and on increased erythrocyte protoporphyrin concentration (Li et al. 1986). Chracateristic plasma fluorescence emission spectrum (maximum at 634 nm) (Poh-Fitzpatrick and Lamola 1976), a low reticulocyte ferrrochelatase activity (Pasanen et al. 1980) and the family history of EPP confirmed the diagnosis. If erythrocyte protoporphyrin concentration was <10 000 nmol/l (normal 200-1010 nmol/l) in patients with photosensitivity but no family history of EPP, a proportion of zinc-chelated protoporphyrin was determined (Orfanos et al. 1989). In symptom-free patients the diagnosis was based on the family history of EPP, decreased reticulocyte ferrochelatase activity and increased erythrocyte protoporphyrin level.

1.3. Porphyria cutanea tarda

Of a total of 85 Finnish patients with PCT who were included for clinical and biochemical studies during 1966-2006, five patients were studied using histopathological methods. Forty-three patients were diagnosed and investigated at Helsinki University Central Hospital. Clinical data from 42 patients were assembled by direct contact with the heads of the Departments of Dermatology and Medicine at the University and other Central Hospitals and using the write-off register for the hospitalised patients in National Board of Health. To improve the validity of the series data for increased excretion of urinary uroporphyrin were collected from two laboratories (United Laboratories Inc, Helsinki and laboratory of Helsinki University Central Hospital) that have facilities in analysing porphyrin excretion using high performance liquid chromatography (HPLC) technique in Finland.

The diagnosis of symptomatic PCT was based on blistering and erosive lesions and/or excessive hyperpigmentation on the sun-exposed skin and increased excretion of urine uroporphyrin with predominance of 7-carboxyl porphyrin (Lim and Peters 1984). In plasma porphyrin fluorescence assay, emission maximum at 617-619 nm supported the diagnosis of PCT (Poh-Fitzpatrick 1980). In symptom-free patients the diagnosis was based on the family history of PCT and the decreased erythrocyte UROD activity (Elder et al. 1989).
plasma fluorescence emission spectrum Chracateristic plasma fluorescence emission spectrum

2. BIOCHEMICAL ANALYSES

2.1. Porphyrin analyses

The erythrocyte and plasma protoporphyrin contents were determined by HPLC (Salmi and Tenhunen 1980; Li et al. 1986). Fluorescence emission spectrum of plasma porphyrins was assayed by direct spectrofluorometry (Schimatzu RF-540, Hitachi F 4010) (Poh-Fitzpatrick and Lamola 1976; Poh-Fitzpatrick 1980). Twenty-four-hour excretion of urine coproporphyrin and uroporphyrin was measured using either spectrophotometry after extraction with ether (With 1968) or HPLC (Sandberg et al. 1982). Faecal coproporphyrin and protoporphyrin were determined either by spectrophotometric assay (Rimington 1958) or by HPLC (Li et al. 1986).

2.2. Enzyme analyses

The lymphocyte PPOX activity was measured by fluorometric assay which measures the oxidation of the non-fluorescent protoporphyrinogen to the fluorescent protoporphyrin (Deybach et al. 1981). One unit of the enzyme activity was defined as the amount of enzyme required to form one nmol protoporphyrin in an hour. The formation of protoporphyrin was determined by fluorescence using Hitachi spectrofluorometer (wavelength of excitation 403 nm, emission 631 nm) and protein concentration was measured using Bio-RAD protein assay (Bio-Rad Laboratories, CA, USA).

The activity of reticulocyte ferrochelatase was measured by radiochemical method (Bonkowsky et al. 1975). Radioactive iron was used in the reaction mixture and the radioactivity of the ^{59}Fe-labelled haem was measured by the Compugamma (LKB-Wallac) sample counter. The enzyme activity was expressed as one pmol ^{59}Fe-labelled haem formed per 10^6 reticulocytes in an hour (Pasanen et al. 1980).

The activity of erythrocyte UROD was assayed by measuring the rate of conversion of uroporphyrinogen I to coproporphyrinogen I (Romeo and Levin 1971; Pasanen et al. 1980). Coproporphyrin formed from coproporphyrinogen by iodine oxidation was measured by spectrophotometry. One unit of the enzyme activity was defined as the amount of enzyme required to form one pmol coproporphyrin in an hour. Protein concentrations were measured using Folin reagent (Lowry et al. 1951).

3. SKIN BIOPSIES

3.1. Tissue samples

In publication I the skin samples were obtained from the back of the hand with signs of old lesions and from the sun-protected gluteal skin in 12 patients with VP who experienced skin fragility. Of eight patients with VP but no skin fragility, the biopsy specimens were taken only from the normal looking back of the hand. In publication V the skin samples were taken from the back of the hand during symptom-free period in eight patients with EPP who experienced photosensitivity and in five cases from the gluteal skin also. In publication VI the skin samples were obtained from the back of the hand and gluteal skin before treatment, at the time of remission and six months later in three patients with PCT treated with chloroquine. In two additional patients with PCT treated with chloroquine, skin biopsies were taken from the back of the hand before treatment and after one year's remission.

3.2. Tissue processing

The formalin-fixed tissue sections were stained with haematoxylin-eosin and PAS as performed routinely. In addition, the samples of VP patients were stained with Alcian blue and those of EPP patients with Congo red and elastin. Direct fluorescence staining of frozen sections was performed with fluorescein isothiocyanate-labeled antibodies to IgA, IgG, IgM and complement.

Samples for EM were fixed overnight or longer in 2.5% glutaraldehyde in ice-cold 0.1mol phosphate buffer (pH 7.2) and postfixed in 1-2 % osmium tetroxide. The specimens were stained with 5% uranyl acetate and lead citrate and examined using JEOL 1200X electron microscope operated at an accelerating voltage of 60 kV.

For immunohistochemistry the paraffin-embedded tissue sections were processed according to the avidin-biotin peroxidase staining method (Wood and Warnke 1981) using StreptABComplex/HRP-Kits (DAKO, Glostrup, Denmark) with primary antibodies. Collagen IV, SAP, kappa and lambda antibodies were obtained from DAKO, laminin from Sigma (St Louis, Mo), versican from Seikagagu (Tokyo, Japan). The samples were incubated with primary antibodies for one hour at room temperature. Aminoethylcarbazole was used as chromogenic substrate. Sections were counterstained with Mayer's haematoxylin. The detailed information of the antibodies are given in publication V.

4. DNA ANALYSIS

4.1. DNA and RNA extraction and cDNA synthesis

Leucocyte DNA was extracted from the venous blood samples with EDTA as an anticoagulant using the QIAmp Blood Kit (Qiagen, Germany) or released from lysates (Higuchi 1989). Total RNA was extracted from Epstein-Barr virus transfected lymphoblast cell lines (Chirgwin et al. 1979; Sambrook et al. 1989). Complementary DNA (cDNA) was synthesised from patients' total lymphoblast RNA by Superscript II RNAse transcriptase (Gibco/BRL, USA) using random hexamers.

4.2. DNA amplification

The polymerase chain reactions (PCR) were performed using 100 ng DNA, 0.2 mM NTPs, 20 pmol of primers and 1U of DNA-polymerase in 50 µl of the enzyme buffer. The primer sequences used for amplification of the cDNA and genomic DNA samples and detailed information of PCR conditions are given in publications III and IV. Radioactive PCR for single strand conformation polymorphism (SSCP) was performed by adding 1 µCi of α^{32}P dCTP (Amersham, Buckinghamshire U.K) in to a reaction mixture.

4.3. Mutation screening using restriction enzymes, SSCP and sequencing

Mutations were identified by restriction enzymes whenever available. Ten µl of PCR product and 20 U of a restriction enzyme in 30 µl of the enzyme buffer were incubated for an hour at the temperature specific for the enzyme used (New England Biolabs, MA, USA).

The resulting fragments were run in 1-4% agarose gel stained with ethidium bromide (Pharmacia Biotech, Uppsala, Sweden) and visualised using UV light. Mutations of the ferrochelatase gene were also analysed using SSCP (Orita et al. 1989). Diluted and denatured PCR products were run in a single-strand separation gel containing 5% or 10% glycerol. Bands were visualised by autoradiography.

The PCR products were purified using Qiaquick PCR Purification Kit (Qiagen, Germany) or Magic PCR Prep Kit (Promega, USA). If PCR was performed with biotinylated primers, purification for single strand sequencing was carried out with streptavidin-coated microbeds (Fluoricon; Idexx, USA). The DNA was sequenced directly by the dideoxynucleotide chain termination method either with Amplicycle Sequencing Kit (Perkin Elmer, NJ, USA) or manually using Sequenase 2.0 Sequencing Kit (USB, Ohio, USA). Each sample included 5 µCi α- ^{33}P-dATP (Amersham, UK). A mutation site was sequenced both with sense and anti-sense primers.

5. TREATMENT WITH HAEM

5.1. Haem administration

In four VP patients infusions of haem arginate (Normosang^{R}, Leiras) 3mg/kg of body weight were initially given daily for four consecutive days, and then once a week for four weeks. Ampoules of haem arginate were diluted in 100 ml physiological saline and given intravenously into a peripheral vein over 15 min. The homozygous VP patient was treated with haem arginate 2 mg/kg for four consecutive days.

5.2. Laboratory measurements

Faecal porphyrins were measured from three samples before haem treatment, after starting the treatment from daily samples for the first 10 days, followed by twice a week sampling up to three weeks after the last infusion. Plasma protoporphyrin and haemopexin, which were analysed by immunodiffusion (Non-Partigen, Hoechst, Germany), were measured before treatment, after four daily infusions, before each weekly infusion and one week after the last infusion. Blood haemoglobin, leukocyte and platelet counts, serum iron, ferritin, transferrin, aspartate aminotransferase, alanine aminotransferase and glutamyltransferase levels were measured simultaneously using routine methods.

5.3. Phototesting

In phototesting a metal halogen lamp (Epolux, 2000 W, Airam, Finland) was used as a source of the light. Emission spectrum of the lamp ranged from 390 to 720 nm with peak at 400-420 nm. The irradiance of the lamp at 400-410 nm varied from 21 to 53 W/m^2 at the distance of 15 cm which was used in the tests. The phototests were performed on the inner side of the forearm before treatment, after four daily infusions of haem, after the second weekly infusion and one week after the last infusion. The shortest time of irradiation capable to induce erythema was taken as the threshold dose.

6. STATISTICAL AND COMPUTER-BASED ANALYSES

Student's t-test was used to compare the means of porphyrin excretions in VP patients with or without skin symptoms. Parameters before and after haem arginate treatment were compared using Student's t-test for paired data.

7. ETHICAL ASPECTS

The study has been approved by the Ethics Committee of the Departments of Medicine and Dermatology of Helsinki University Central Hospital.

RESULTS AND DISCUSSION

1. PREVALENCE AND INCIDENCE OF CUTANEOUS PORPHYRIAS IN FINLAND

1.1. Variegate porphyria

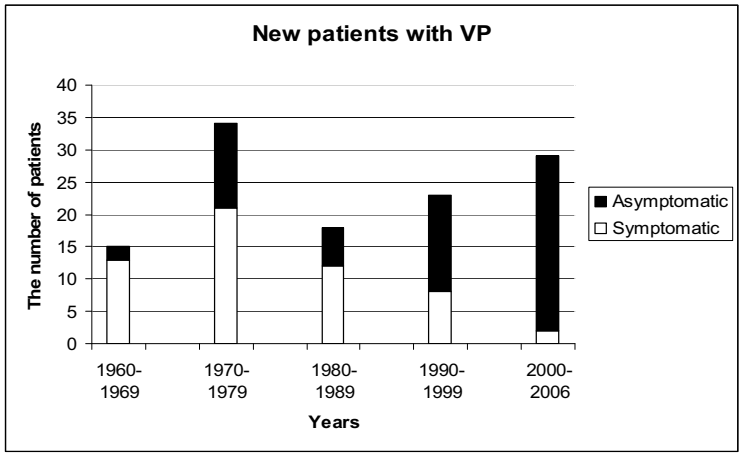


Figure 9. The new patients with VP identified from 1960 to 2006.

The prevalence of VP estimated in 1978 was based on the analysis of 48 patients and equalled 1.3 per 100 000 Finnish population older than 14 (Mustajoki 1980). A total of 73 VP cases were diagnosed in Finland in 1989 (Kauppinen and Mustajoki 1992). During the last two decades the majority of the new patients have been asymptomatic (Figure 9). Since the 1960's altogether 156 patients with VP have been revealed in 22 families. At the end of 2006 108 patients were alive. Thus, the current prevalence of VP including both symptomatic and asymptomatic cases is approximately 2.1:100 000 in the Finnish population of 5.2 million indicating still a slight increase in the number of patients because of genetic testing (von und zu Fraunberg et al. 2002). Of 70 new patients diagnosed since 1980, 22 were symptomatic (Figure 9) providing the incidence of 0.2:1 000 000 calculated in the population older than 14. The increase in the number of patients since 1970's reflects the activity of finding new cases by means of biochemical analyses and mutation screening. Moreover, contemporary information and knowledge about porphyrias among physicians have contributed to the number of new patients.

Although several VP series have been published in Western Europe (Whatley et al. 1999; D'Amato et al. 2003; Lecha et al. 2006), VP is still far more frequent in Finland than in other countries outside South Africa (Elder et al. 1997; Wiman et al. 2003b). This may result from a founder effect since the major mutation characterised in 11 families follows the settlement of the Finnish population during the last 500 years in the southern-western part of the country (von und zu Fraunberg and Kauppinen 2000). Although their pedigrees could not be united and haplotyping analysis was not performed, they most likely have a common ancestor in Finland. Interestingly, the Finnish major mutation was also reported from France and in USA (Frank et al. 1998b; Whatley et al. 1999). In Sweden approximately 80 VP patients have been diagnosed in 28 unrelated families resulting in prevalence of 1:100 000 (Wiman et al. 2003b).

1.2. Erythropoietic protoporphyria

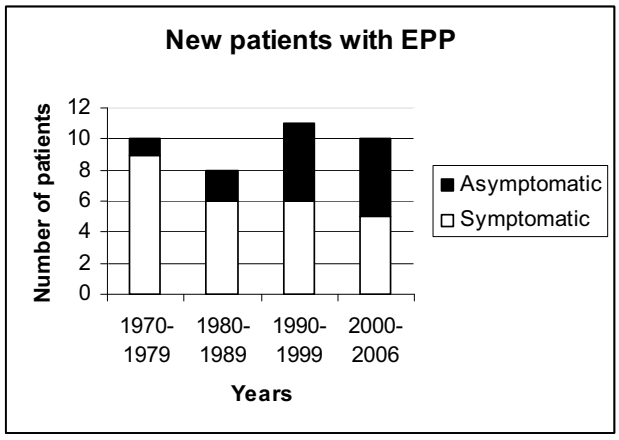


Figure 10. The new patients with EPP identified from 1970 to 2006.

Previously only one Finnish EPP family including one patient with a manifest disease and her five symptom-free family members has been described (Hopsu-Havu et al. 1973). Although EPP has been screened since 1970's among patients with photosensitivity in the dermatological departments of University Central Hospitals (Hopsu-Havu et al. 1973), the number of EPP patients is low in our country. To date we have enrolled 45 EPP patients belonging to 15 families, of whom 39 patients were alive at the end of 2006. Thus, the prevalence of EPP in Finland is approximately 0.8:100 000 including both symptomatic and asymptomatic patients. If only symptomatic patients (n=23) are included as in the surveys from other countries, the estimated prevalence is 0,4:100 000. This is lower than reported from the Netherlands (1:75000) and the United Kingdom (1:136 000) (Todd 1994; Holme et al. 2006b) but comparable to that reported from Sweden (0,5:100 000) (Wiman et al. 2003a). Approximately one symptomatic patient has been diagnosed every second year providing the incidence of 0.1:1 000 000 but in the majority of the families new asymptomatic individuals have been identified by mutation screening or biochemical analyses (Figure 10).

1.3. Porphyria cutanea tarda

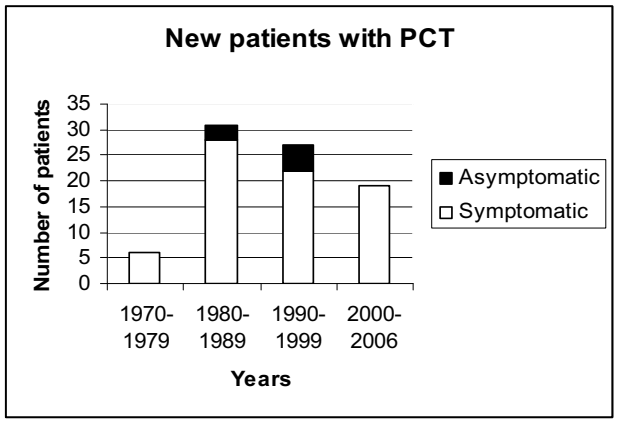


Figure 11. The new patients with PCT identified from 1970 to 2006.

During 1972-2006, 85 patients with PCT were diagnosed in Finland (Figure 11). Of the patients, 81 subjects had skin symptoms, three subjects were asymptomatic family members identified only by enzyme or mutation analysis and one case had biochemical chracteristics of PCT, but no skin symptoms. The data indicates that two to three new patients with PCT have been diagnosed in Finland yearly during the last 2.5 decades. Sixty three patients were alive at the end of 2006. According to these figures the prevalence of PCT to date is 1.2:100 000 in the Finnish population of 5.2 million and the incidence 0.5:1 000 000 per year. The estimated prevalence of PCT in the capital region including 1.5 million inhabitants is 1.2:100 000 on the bases of 18 living patients which is comparable to the figures of the whole country. These numbers are considerably lower than elsewhere in Western Europe, where the prevalence of PCT ranges from 1: 1000 in the former Czechoslovakia (Martasek et al. 1987) to 1: 25 000 in the United Kingdom (Elder 1990). Incidence of PCT in the United Kingdom (Elder 1998) has been estimated at 2-5: 1 000 000 per year, which is more than 4-fold compared to that of Finland.

The causes for the low incidence of PCT in Finland may be related to varying presence of associating or genetic factors. Hepatitis C infection is uncommon in Finland in contrast to the Southern Europe (Cruz-Rojo et al. 2002). Increasing consumption of alcohol is comparable to that the other European countries (Helasoja et al. 2007), which suggests that alcohol is not the major factor at the epidemiological level**.** Moreover, the frequency of *HFE* mutations in the Finnish population is comparable to that in Europe and the United States of America (US) (Parkkila et al. 2001). Currently unidentified genetic factors, which may explain the low morbidity of PCT in Finland, cannot be excluded.

In Finland the occurrence of subtypes of porphyria differs from that of other countries. AIP is the most common porphyria in Finland (Kauppinen and Mustajoki 1992), Sweden (Andersson et al. 1995) and Germany, while PCT is the most common form of porphyria in US and Southern Europe (Elder 1998). In Finland the prevalence of VP is 2-fold compared to that of PCT (2.1 vs. 1:100 000), but the estimated incidence of PCT exceeds 2-fold that of VP (0.5 vs. 0.2:1 000 000 per year). The lower incidence number of VP is probably attributed to a few symptomatic patients diagnosed during last decades. EPP is the second commonest cutaneous porphyria in many countries after PCT**,** which may be caused by infrequency of VP in those countries In Finland EPP is clearly less frequent in comparison to other types of cutaneous porprhyria. To date no patients with CEP and HCP have been found in Finland.

2. DIAGNOSTICS OF CUTANEOUS PORPHYRIAS

(Article I, IV and V)

2.1. Biochemical and mutation analyses of VP

The results of the biochemical analyses in 91 patients with VP tested in remission are presented in Table 9 and those who have experienced skin symptoms are presented in Table 10. Plasma porphyrin fluorescence assay showed the characteristic peak at 626 nm in 21 of 30 patients examined. In all symptomatic patients (n=19) the plasma fluorescence scanning was positive. Two of the patients with a positive result and all the patients with negative plasma porphyrin fluorescence (n=9) were asymptomatic family members screened by DNA analysis and their faecal and urinary porphyrin excretions were also within the normal range. The results indicate that plasma porphyrin scanning detects reliably the symptomatic patients but it is not sensitive enough to identify asymptomatic family members. In the assays of the urinary and faecal porphyrins the typical findings were the increased excretions of faecal protoporphyrin and urinary coproporphyrin (Article I). The mean faecal protoporphyrin level was 3.5-fold compared to the normal upper limit exceeding distinctly that of coproporphyrin which was only slightly increased (1.6-fold) (Table 9). The means of urinary coproporphyrin and uroporphyrin levels increased in a same proportion compared to the normal upper limits (2.7- and 2.5-fold respectively), but the total increase of coproporphyrin level exceeded distinctly that of uroporphyrin which remained near to the normal range. In 40% (30/75) of the patients urinary coproporphyrin excretion was within the normal range and correspondingly in 49% (32/65) of the patients of urinary uroporphyrin excretion was within the normal range or only slightly increased (<60 nmol/d). Urinary coproporphyrin level more than 1000 nmol/d was associated with an increased risk of clinical symptoms (von und zu Fraunberg et al. 2002).

The patients with skin symptoms had a higher excretion level of urinary and faecal porphyrins compared to that of all patients measured (Table 9 and 10). Urinary coproporphyrin (3.9-fold) excretion exceeded that of uroporphyrins (3.6-fold) and faecal protoporphyrins (4.8-fold) that of coproporphyrins (2.4-fold), respectively (Table 10). Excretion of urinary porphobilinogen and was normal in 31% of the cases (10/32) and only slightly increased in the others. The increased excretion of faecal protoporphyrin was found in all patients with skin symptoms except one who was symptom-free at the time of the examination. He had experienced skin symptoms for several years earlier suggesting that normal faecal excretion of protoporphyrin predicts freedom of symptoms (von und zu Fraunberg et al. 2002). These results indicate that after a mutation has been identified in the asymptomatic family member, biochemical tests should be done in the adult individual to predict risk for clinical manifestations.

The results demonstrated that the constant finding in patients with VP and the current skin symptoms was the typical plasma porphyrin fluorescence and increased level of faecal protoporphyrin excretion. The plasma fluorescence at 626 nm is specific for VP and the test covers also patients with acute symptoms and commonly also those, who have had symptoms earlier. Plasma porphyrin fluorescence may be positive in symptom-free patients (Da Silva et al. 1995) and thus, it cannot be used solely in the diagnostics of skin symptoms. Furthermore, faecal analysis alone does not exclude the secondary increase of protoporphyrins caused by intestinal flora and haemoglobin in the faeces (Moore et al. 1987). In conclusion, the diagnosis of symptomatic VP should be based on both of these assays before the causality of the skin symptoms and abnormal porphyrin profile can be established. The urinary porphyrin analysis is not diagnostic but rather an additional investigation in VP to exclude PCT.

The mean activity of lymphocyte PPOX was approximately 60% of the normal level (Table 11). However, in six (17%) of the 35 patients the enzyme activity was either normal or near the upper normal limit (> 3.0 nmol proto/h/mg protein) overlapping with the levels of healthy family members. Thus, the PPOX activity is insufficient as a sole investigation for the diagnosis of VP. Of note, 18 (70%) of 25 patients with VP had also a low erythrocyte FECH activity (mean 3.7 pmol/haem/h retic), which may be a secondary phenomenon to the deficient PPOX activity or it may reflect the low normal FECH activity reported among healthy controls due to a polymorphism in the FECH gene (Gouya et al. 2002).

Mutation screening in the PPOX gene can be used to confirm the diagnosis, but a typical biochemical profile of VP is mandatory in order to prove the causality of skin manifestations to VP. A total of nine mutations have been identified among Finnish patients with VP (von und zu Fraunberg and Kauppinen 2000; von und zu Fraunberg et al. 2001). The major mutation (R152C) in the western part of Finland and the knowledge about the region from which the families originate facilitates DNA analysis in our country. However, the biochemical abnormalities must always be detected in an index case before sequencing of the PPOX gene is reasonable. Thereafter, the information about the family specific mutation can be used in family screening. The purpose of DNA analysis is to find the patients, who are at risk for acute attacks and should avoid precipitating factors. If a patient has skin symptoms, plasma porphyrin fluorescence assay and analysis of urinary and faecal porphyrins are the methods of choice.

2.2. Biochemical and mutation analyses of EPP

The increase of erythrocyte protoporphyrin concentration was shown to be the principal abnormality in EPP (Table 9). The mean value of erythrocyte protoporphyrins (40 968 nmol/l) in the patients with skin symptoms was 40-fold compared to the normal, but there was a wide variation in the levels (Table 18). The lowest value of erythrocyte protoporphyrin associated with skin symptoms was 5-fold (4847 nmol/l) the normal (Table 18). Of note, at the time of diagnosis in this case erythrocyte protoporphyrin concentration measured by semiquantitave method exceeded 60–fold the normal (data not shown). In asymptomatic patients the erythrocyte protoporphyrin levels were constantly on the upper limit of the reference value or slightly increased exceeding up to 3-fold the normal (Table 18). The mean of erythrocyte protoporphyrin concentrations among symptomatic patients was 25-fold compared to that of asymptomatic patients (mean 40 968 vs. 1668 nmol/l, Table 18).

The plasma porphyrin fluorescence assay studied in 20 patients with skin symptoms showed a characteristic peak at 634 nm in all of them (Table 9). The excretion of urinary uroporphyrin was only slightly increased in five (6%) of the 18 patients analysed and that of urinary coproporphyrin was within normal range (Table 9). The mean of faecal protoporphyrin excretion was slightly (2.7-fold) increased among symptomatic patients (mean 354 nmol/g dry weight). Of note, it was normal in five symptomatic patients.

Erythrocyte FECH activity was decreased in all patients with the skin symptoms and the mean activity was 13% of the normal (Table 11) mimicking the recessive pattern of inheritance. In six cases, the enzyme activity was measured from the lymphocytes. In DNA analysis four novel mutations (751delGAGAA, 1122Tdel, C286T, C343T) were identified in the FECH gene in 10 patients of the four families studied (IV). All symptomatic patients had dramatically increased levels of erythrocyte protoporphyrins (Table 18), and thus, the mutation analysis provided no additional information for the clinical diagnosis of EPP in these patients. Twelve asymptomatic patients with EPP, whose blood protoporphyrin levels were only slightly

increased (mean 1.7-fold increase compared to normal, Table 18), could be diagnosed as carriers of a mutation. Of note, one of the mutations, 1122delT, was a de novo mutation.

In symptomatic patients with EPP the biochemical diagnosis is based on the high concentration of erythrocyte protoporphyrins usually more than a 5-fold increase compared to normal. If the increase is less than 10-fold, plasma porphyrin spectrum should be performed to confirm the diagnosis of EPP. Less than a 5-fold increase can be also related to iron deficiency, lead intoxication or homozygous porphyrias other than EPP (Zaider and Bickers 1998) and thus, the ratio of the free and zinc chelated protoporphyrin should be measured (in EPP the ratio <1) before the diagnosis of EPP can be established (Orfanos et al 1989). Biochemical methods poorly recognize asymptomatic patients carrying the affected gene, and thus, mutation analysis can be used to identify these family members if necessary for genetic counselling but typical clinical symptoms and accurate biochemical findings are usually sufficient to confirm EPP.

2.3. Biochemical analyses of PCT

In the patients with PCT the biochemical hallmark was the increased excretion of urinary uroporphyrin and 7-carboxyl porphyrins (Table 9). The excretion of urinary uroporphyrin varied from 15 to 350 (mean 110)-fold compared to that of normal. The excretion of 7-carboxyl porphyrin was increased in all patients with PCT analysed ranging from 2 to 316-fold compared to that of normal. In addition, urine contained excess of 6- and 5-carboxyl porphyrins but the level of 7-carboxyl porphyrin was higher in all individuals examined. On the whole, the levels of carboxylporphyrins decreased linearly compared to the level of uroporphyrin. Level of urinary coproporphyrin was increased (2-fold) in the majority of cases, but it was normal in 24% (17/71) of the patients studied. Compared to VP, the increase of urinary coproporphyrin excretion was less extensive.

The mean excretion of total faecal coproporphyrin was within the normal range, but it was mildly increased in 10 (22 %) of the 45 patients studied up to 3-fold (mean 179, range 104-328 nmol/g dry weight). Presence of faecal isocoproporphyrin which is dehydroisocoproporphyrin metabolized by bacteria in the gut, is informative for the diagnosis of PCT (Moore et al. 1987; Cooper et al. 2005), but it was not analysed in our series separately. Faecal protoporphyrin level normal in the majority of cases, but in 5 (12%) of 42 patients (mean 260 nmol/g dry weight, range 190-344), it was increased 2-fold indicating induced metabolism of porphyrins even beyond UROD. In plasma porphyrin fluorescence assay, maximum at 617-619 nm

Scanning of plasma fluorescence emission spectrum showed a peak at 617-619 nm in all patients examined (n=43). This is not specific for PCT, because AIP and HCP give a similar plasma fluorescence pattern. The sensitivity of plasma fluorescence was 100% in symptomatic patients.

Erythrocyte UROD activity was decreased in 26 of 53 patients (49%) suggesting type II PCT. Only two of the symptomatic patients were from the same family (father and son). Based on enzyme analysis the proportion of type II PCT in Finland is 2-fold compared to those reported from Hungary (23%, based on enzyme analysis), Italy (26%, based on enzyme analysis), Denmark (25%, based on mutation analysis) and Spain (26%, based on mutation analysis) (Koszo et al. 1992; Tavazzi et al. 2002; Bygum et al. 2003; Mendez et al. 2007). Our results are similar to those reported from Germany (50%, based on enzyme analysis), Spain (37%, based on enzyme analysis) and Chile (50%, based on mutation analysis) (Doss et al. 1991; Cruz-Rojo et al. 2002; Poblete-Gutierrez et al. 2004).

The mutation analysis revealed a C304T mutation in the UROD gene in a patient with PCT and his asymptomatic daughter. Since both of them had normal erythrocyte UROD activity, PCT was classified as type III. Currently, the clinical relevance of the mutation analysis is uncertain since the biochemical abnormalities are essential in the diagnosis of PCT. In asymptomatic family members, the significance of a carrier status of a mutation is even more troublesome to interpret. No information is available about the risk of developing symptomatic PCT in mutations carriers. Mutation screening may be an additional screening method for the measurement of the UROD activity in order to classify the subtypes of PCT (Bygum et al. 2003; Mendez et al.2007).

Table 9. Biochemical analyses of patients with cutaneous porphyria at the time of diagnosis during 1966-2006

Biochemical Analysis	Normal range	VP Total number of patients:156			PCT Total number of patients: 85			EPP Total number of Finnish patients: 48		
		Patients	Mean	Range	Patients	Mean	Range	Patients	Mean	Range
U-PBG	<9 umol/l	N=69	19	1-104						
U-DALA	<34 umol/l	N=67	42	2-133						
dU-Uroporphyrin	<36 nmol	N=65	93	6-414	N=75	3946	535-12571	N=18	32	2-90
7-Carboxyl porph	<15 nmol				N=57	1302	37-4740			
6-Carboxyl porph	<7 nmol				N=53	340	4-2201			
5-Carboxyl porph	<6 nmol				N=42	273	10-2000			
dU-Coproporphyrin	<230 nmol	N=75	620	5-3643	N=71	464	39-1765	N=23	71	13-50
F-Coproporphyrin	0-100 nmol/g dry weight	N=82	164	2-888	N=45	67	1-328	N=21	9	1-40
F-Protoporphyrin	0-130 nmol/g dry weight	N=83	455	12-1431	N=42	70	2-344	N=25	302	15-1439
B-Protoporphyrin	250-1050 nmol/l							N=39	27650	1000-102568
P-Fuorescence		N=21	+626 nm		N=43	+617-619 nm		N=20	+634 nm	
		N=9	-					N=5	-	
Total number of patients with diagnostic laboratory analysis done		N=91			N=78			N=37		
No biochemical results available, the diagnosis has been done by pedigree analysis, mutation analysis solely or by previous clinical manifestations		N=65						N=11		
No biochemical results available, the diagnosis has been done in other hospitals by dermatologists					N=8					

Table 10. Biochemical analyses of VP patients with skin symptoms[1]

Biochemical analysis	Normal range	VP Total 44 patients with skin symptoms		
		Patients	Mean	Range
U-PBG	< 9 umol/l	N=32	23	4-104
U-DALA	< 34 umol/l	N=31	50	7-130
dU-Uroporphyrin	< 36 nmol	N=29	130	20-414
dU-Coproporphyrin	< 230 nmol	N=32	893	90-3643
F-Coproporphyrin	0-100 nmol/g dry weight	N=36	241	8-888
F-Protoporphyrin	0-130 nmol/g dry weight	N=38	621	47-1431
P-Fluorescence	626 nm	N=13	+	
Total number of patients with diagnostic laboratory analysis done during skin symptoms only		N=38		
No biochemical results available in remission and the diagnosis has been done during an acute attack		N=6		

[1]The biochemical analyses have been performed in remission from of acute attacks

Table 11. The results of enzyme analyses and the number of patients with a mutation in VP, PCT and EPP

	Normal	VP Total no of patients: 156			PCT Total no of patients: 85			EPP Total no of patients: 48		
		Patients	Mean	Range	Patients	Mean	Range	Patients	Mean	Range
Ly-PPOX	3.9-6.0 nmol Protoporph/h/mg prot	N=34 N=1[1]	2.9 0.5[1]	1.6-6.2						
E-FECH	6.0-22.0 pmol Haem/h/10^6 retic	N=25	6.0	1.6-17.5				N=33	2.0	0.2-5.4
Ly-FECH	>1 nmol/h/mg							N=6	0.6	0.3-1.0
E-UROD	65-100 pmol Coproporph/h/mg prot	N=13	76	59-96	N=63 Type I[2] N=36 Type II[3] N=26 Type III[2] N=1	 79 48 68	 65-140 23-60			
Positive DNA analysis		N=82			Type II N=5 Type III N=2			N=36		

[1]Homozygous patient with VP; [2]normal UROD activity in erythrocytes; [3]low UROD activity in erythrocytes;

3. CLINICAL MANIFESTATIONS

3.1. Variegate porphyria

3.1.1 Occurrence of the symptoms

Of the total of 156 VP patients (Table 12), 90 (58%) were women and 66 men (42%) and full clinical information was available in 112 cases (Table 13 and 14). Fifty-four percent (61/112) of them experienced clinical symptoms (Table 13). Of symptomatic patients, 64% had acute attacks and 67% skin symptoms, respectively. However, the occurrence of acute attacks decreased dramatically from 51% to 18% during the last decades (Table 12). The occurrence of skin symptoms decreased only slightly from 44% to 30% at the same time. The frequency of photosensitivity as the only clinical sign was stable during the follow-up before or after 1980 (22% vs. 21%), but acute attacks became rare (29% vs. 7%) during the last two decades. The number of patients having both acute attacks and photosensitivity decreased substantially (22% vs. 11%), but still 30% of the symptomatic patients have both skin symptoms and acute attacks (Table 13).

Table 12. The clinical manifestations of VP patients during the follow-up

	Year of diagnosis and number of the patients					
	Before 1980		1980-2006		Total	
	N	%	N	%	N	%
Acute attacks only	16	29	4	7	20	18
Photosensitivity only	12	22	11	19	23	21
Photosensitivity and acute attacks	12	22	6	11	18	16
No symptoms	15	27	36	63	51	46
No information about clinical manifestations[1]	21		5		26	
No information about acute attacks/skin symptoms[1]	4/7		0/0		4/7	
Age < 15 years[1]	-		7		7	
Total	87		69		156	
Mutation known	21		60		81	

[1]Excluded from the percentages; the majority of the patients were diagnosed using pedigree analysis

The penetrance rates of VP have varied from series to series (von und zu Fraunberg et al. 2002; Hift et al. 2004b;Whatley et al. 1999). The frequency of acute attacks has varied from 4% to 38% and skin symptoms from 40% to 70%, respectively, depending on the ratio of asymptomatic and symptomatic patients and active mutation screening in these families. If careful mutation screening has been performed as described in one South African (Hift et al. 2004b) or several Finnish families (von und zu Fraunberg et al. 2002), the clinical manifestations occur in 40 % of the mutation carriers. Current results suggest a descending tendency for penetrance of VP (37 %).

Table 13. The clinical manifestations of symptomatic patients with VP during the follow-up

	Year of diagnosis and number of the patients					
	Before 1980		1980-2006		Total	
	N	%	N	%	N	%
Acute attacks	28	70	11	48	39	64
Photosensitivity	24	60	17	81	41	67
Subgroups						
Acute attacks only	16	40	4	19	20	33
Photosensitivity only	12	30	11	52	23	38
Photosensitivity and acute attacks	12	30	6	29	18	30
Total	40		21		61	

Of 61 symptomatic patients, 30% experienced both symptoms, 33% only acute attacks and 38% skin symptoms respectively (Table 13)**.** Among symptomatic patients the occurrence of acute attacks has decreased from 70% to 48% during the last two decades and thus, the proportion of skin symptoms has increased from 60% to 81%, respectively. These numbers are comparable to other series (Whatley et al. 1999) indicating that acute attacks have become less frequent especially if acute porphyria has been diagnosed at the asymptomatic phase and the patients can avoid precipitating factors (Kauppinen and Mustajoki 1992).

In six patients who had both skin symptoms and acute attacks after 1980 the skin symptoms were present before the acute attacks manifested. Of the 21 patients diagnosed after 1980, nine symptomatic patients (45%) had no family history of VP. Therefore, lack of family history does not exclude acute porphyria. Both symptomatic and asymptomatic patients with VP were identified later among family members in five of these cases. The other four were still single patients in the family, but no extensive mutation screening has been performed.

Table 14. Frequency of skin symptoms and acute attacks by gender and follow-up periods

Symptom	Male				Female			
	Before 1980		1980-2006		Before 1980		1980-2006	
	N	%	N	%	N	%	N	%
Acute attacks only	4	20	1	4	12	34	3	9
Photosensitivity only	5	25	4	17	7	20	7	21
Photosensitivity and acute attacks	4	20	0		8	23	6	18
No symptoms	7	35	18	78	8	23	18	53
No full information[1]	14		4		18		1	
Children[1]	-		5		-		2	
Total number of patients	34		32		53		37	

[1]Excluded from the percentages

Of the 61 symptomatic patients 70% (43) were women and 30% (18) men. No difference in gender was observed in the frequency of skin symptoms, but the low number of acute attacks was especially obvious among men who can avoid most of the precipitating factors. In women the menstrual cycle has been the commonest precipitating factor (Kauppinen and Mustajoki 1992)

3.1.2. Skin symptoms

Of 41 patients detailed information about skin symptoms was available (Table 15). The onset of the photosensitivity varied from 14 to 53 years (mean 23 years). In five cases (11%) skin symptoms did not manifest until the age of 30. The majority of the patients were women (30 women, 68%, vs. 12 men, 32%). The predominance of women has been explained by the presumption that women are more vulnerable for minor trauma in their daily work compared to men (Mustajoki 1980).

Ninety five percent of the patients experienced photosensitivity which manifested as excessive fragility resulting in erosions and blistering on the sun-exposed skin (Table 15). Skin fragility occurred constantly on the backs of the fingers and hands and to lesser extent on the face and the arms despite chronic sun exposure. Less than half of the patients experienced blisters on the back of the hands, which probably was a sign of more severe photosensitivity. Blisters never occurred as a sole symptom, but always together with erosions after minor trauma. In a half of the patients the skin symptoms showed seasonal variation increasing in late summer and autumn. However, a similar proportion of the patients reported skin fragility throughout the year. Typical skin symptoms in the selected group, which was studied for histological changes, were comparable to those of the total group, and thus, the results gained from the selected group were representative for more general conclusions (Table 15).

Table 15. The characteristics of skin symptoms in heterozygous patients with VP

	Selected group in the histological studies[1]		All patients with the detailed information about skin symptoms	
Symptom	N=12	%	N=41	%
Photosensitivity				
Fragility	12	100	39	95
Erosions				
Hand	12	100	37	90
Arm	5	42	14	34
Face	7	50	17	41
Foot	3	25	7	17
Leg	1	8	1	2
Blisters[2]				
Hand	5	42	19	46
Face	0		1	2
No seasonal variation	5	42	24	59
Hypertrichosis	5	42	14	34
Hyperpigmentation	1	8	8	20

[1]Article I

Previously skin symptoms which were studied in 53 Finnish patients with VP have been considered mild and less common (45%) (Mustajoki 1980) than in those from the other countries (80%) (Eales et al. 1980; Whatley et al. 1999) where the sun exposure is stronger. Only 17% (4/24) of Finnish patients had contacted a physician because of their skin symptoms before 1980, which is comparable to our present results (17%, 7/41). The recent series including 28 adult family members with South African founder mutation demonstrated that 40% of them experienced skin symptoms which located in the hands in 90% of patients (Hift et al. 2004b). The site of skin symptoms in the Finnish patients was comparable to the results obtained from South African patient data: the dorsal side of the hand was affected constantly and the face in less than half of the cases. The hands are probably affected more commonly than the face because of the exposure to minor trauma (Mustajoki 1980).

Although clinical manifestations of the skin symptoms have remained the same throughout the five decades in Finland, early diagnostics may reveal more patients with milder skin symptoms and genetic counselling may diminish the severity of the skin symptoms when the patients are guided to protect their skin with clothes. Currently the majority of the patients with VP have been diagnosed using biochemical or mutation screening methods at the asymptomatic phase.

Of 41 patients with skin symptoms, seven patients (17%) had a history of acute transient light eruption in the early summer. Since light eruptions are as common as in 20 % of normal population (Jansen and Karvonen 1984), they may not be directly associated with porphyria in these patients, although it has been suggested (Day 1986).

Hypertrichosis was present in the face skin in the third of the patients with VP (Table 15). Only two of the 14 patients were men indicating that hormonal factors may influence the presence of hypertrichosis as described in that of other origin (Ramos-e-Silva et al. 2008). It was mostly mild lanugo-type together with long hairs locating on the cheeks, periorbital and temporal areas.

Hyperpigmentation occurred in the fifth of the patients, mainly in the face and cheeks, which could be difficult to distinguish from melasma and hyperpigmentation related to systemic diseases (Ramos-e-Silva et al. 2008). It was the only presentation of the skin symptoms in two patients, and the association of hyperpigmentation with porphyria was supported by the highly increased excretion of coproporphyrins in the urine (>1000 nmol/d), which predicted symptoms of porphyria among patients with VP (von und zu Fraunberg et al. 2002) The presence of hypertrichosis and hyperpigmentation did not seem to be associated with the severity of the skin fragility.

Hypertrichosis and hyperpigmentation are much rarer (up to 30%) than photosensitivity in South African patient series (Eales et al. 1980; Hift et al. 2004b) but they can easily be neglected even by questionnaires. Patients with VP seldom complain of them spontaneously.

3.1.3. The homozygous patient

(Article III)

The homozygous patient was followed clinically and biochemically for 20 years. He developed a severe bullous skin disease post partum followed by increased fragility and cheloid-like scarring (Mustajoki et al. 1987). Up to five years of age he also experienced acute photoreactions. Severe blistering and fragility of the sun exposed skin have continued during the follow-up in the summer and autumn. Skin lesions were complicated by deep erosions and bacterial infections. The development of new scars declined but they were still abundant

including dorsal parts of the hands, ears, neck, and the scalp accompanied by partial alopecia (Figure 12). Radial deep furrows around the mouth developed gradually. Four subsequent haem arginate infusions corrected only partially porphyrin metabolism for a few days (Article II).

The fingers of the patient were markedly shortened and thickened with flexion impairment. Early closure of the phalangeal epiphyses was detected by radiographs. Mental status, EEG and CT of the head remained normal, but sensory polyneuropathy was shown in nerve conduction and sensory evoked potential studies especially in the upper extremities. Fine motor-coordination disturbances were accompanied by minor verbal and visuospatial deficiencies. Raised intra-ocular pressure responding to a beta-blocking agent and myopia were detected at the age of six (vision 0.7). The patient never experienced acute attacks indicating that the clinical manifestations were chronic and neurological and cutaneous in origin.

Since age of 20 serum creatinine level was constantly increased accompanied by haematuria, proteinuria and increase of blood pressure (BP 140/90) indicating a renal failure. Renal biopsy revealed sclerotic changes in glomeruli. Using immunofluorescence techniques a strong IgA and moderate C3 depositions were found in mesangium and paramesangium areas confirming IgA-nephropathy. Renal failure progressed to oliguria and the patient was treated with peritoneal dialysis for the last three years before kidney transplantation. Cholecystectomia was performed before transplantation because of cholelithiasis. Traditional operation techniques and filters in the lamps of the operating room were used successfully without phototoxic complications.

Like the other patients with homozygous VP, our patient had severe photosensitivity from the early childhood in addition to deformities of the hands (Hift et al. 1993). Scar formation is a constant phenomenon among these patients (Table 4), but scarring alopecia has been described earlier only in one patient (D'Alessandro Gandolfo et al. 1991). Sensory neuropathy has occurred only in one additional patient earlier (Hift et al. 1993). Our patient had no mental retardation and convulsions, which have been present in half of the cases reported (Table 4). Acute attacks have been described in one patient with an adult onset of clinical manifestations (Corrigall et al. 2000) and in the other patient with uncertain diagnosis of homozygous VP (Coakley et al. 1990) but not in the characteristic patients with severe photosensitivity since the childhood (Table 4).

Renal failure has not been reported in the other patients with homozygous VP (Table 4) but it is not rare finding among patients with acute porphyria (Kauppinen and Mustajoki 1992; Andersson et al. 2000). The histology of renal biopsies from these patients varies from glomerulonephritis to tubular fibrosis, but IgA nephropathy has not been documented among other patients with porphyria.

All our patient's relatives have been symptom-free throughout the follow-up years. Only one of 12 patients, who have the same mutation (I12T) at the heterozygous state, has experienced two acute attacks at the time of the diagnosis but no skin symptoms. She belonged to another family and the pedigrees could not be united suggesting that other modifying genes interfere with the phenotype. These results demonstrated that the I12T mutation is clinically milder at the heterozygous state compared to the other mutations found in Finland (von und zu Fraunberg et al. 2002).

Figure 12. Skin manifestations of homozygous VP patient (on the courtesy of the patient)

3.1.4. Treatment of haem arginate in skin symptoms of VP

(Article II)

Biochemical effects

After initial four daily doses of intravenous haem arginate, the excretion of faecal protoporphyrin (mean 579 nmol/g dry weight) fell to an almost normal level (mean 123 nmol/g dry weight), and that of coproporphyrin (mean 162 nmol/g dry weight) to the normal level (mean 21 nmol/g dry weight) in all patients. During the period of four weekly infusions of haem the excretions of faecal porphyrins initially fell in three patients but returned within a week to almost the pretreatment level. In one patient the weekly infusions had no effect on protoporphyrin excretions. The excretion of faecal porphyrins after treatment remained at the pretreatment levels in all patients. Similarly, the plasma protoporphyrin concentrations decreased initially significantly but increased to the pretreatment levels during weekly infusions.

The biochemical effect of haem arginate infusion did not differ from that described in the previous series in which the compound was tested in acute attacks of patients with VP (Mustajoki et al. 1989).

Clinical effects

In phototesting, in which the source of light emitted radiation at visible region peaking at 400-420 nm (covering the Soret band), the time for the erythema threshold at the initial phase of treatment in patients with VP was lower than in healthy controls. These results are in concordance with those reported earlier (Mustajoki 1980) suggesting that patients with VP may also have a predisposition to transient photoreactions on the basis of phototoxicity. During the haem arginate treatment no constant improvement in photoreactivity could be found, although the levels of plasma protoporphyrin temporarily decreased. The skin symptoms disappeared in one patient only but remained unchanged in the others indicating a poor clinical effect. The only side effect was a mild thrombophlebitis in one patient. Thrombophlebitis related to infusions of haem arginate has been reported earlier in occasional cases (Mustajoki and Nordmann 1993).

The results of the present study demonstrate that daily infusions of haem can produce transient biochemical remission as in the use in acute attacks (Mustajoki et al. 1989) but the weekly infusions do not maintain the plasma protoporphyrin concentration and the faecal porphyrin content at the low level. More frequent infusions of haem e.g. twice a week are probably required to obtain long-lasting biochemical remission in VP but frequent infusions of haem may induce haemosiderosis. Transient normalisation of porphyrin excretion and plasma protoporphyrin concentration had no effects on phototesting or clinical symptoms. To date no other series in which haem arginate was tested in photosensitivity have been published.

3.2. Erythropoietic protoporphyria

3.2.1. Skin symptoms

Of 45 patients with EPP 25 individuals experienced photosensitivity (Table 16). The others were symptom-free family members or their clinical manifestations were unknown. In the whole group more than half of the patients were male. Among symptomatic patients the sex distribution was similar. The onset of photosensitivity varied from 6 months to 23 years of age (mean 4.6 years). In all except one patient it became manifest by 10 years of age. In 17 (68%) patients the onset was in the early childhood before 6 years of age.

Table 16. Clinical data of patients with EPP

	Male		Female		Total	
	N	%	N	%	N	%
Symptomatic	14	56	11	44	25	64
Onset at <6 years old	9	36	8	32	17	68
Asymptomatic	9	64	5	36	14	36
No information of clinical manifestations[1]	4		2		6	
Total number of patients	27	60	18	40	45	

[1]Excluded from calculations

The details of the skin symptoms are presented in Table 17. The skin symptoms occurred within fifteen minutes to six hours after sun exposure and usually lasted 1 to 2 days. The exposure time needed for provoking skin symptoms varied intra-individually. The main symptom was a stinging or burning pain (96 %) in the exposed skin followed by swelling (Table 17, Figure 13). Itching was reported by one fourth of the patients, but only in two patients itching was more intensive sensation than pain. In addition to the characteristic swelling three patients experienced superficial erosions in the bridge of the nose. One of them also had petechial reactions after prolonged sun exposure (Figure 14). Chronic skin changes including thickening of the knuckles and furrows in the lips were found in nine patients. The youngest of them was four years old.

Table 17. The clinical characteristics of photosensitivity in EPP patients

Sign	Number of symptomatic patients	%	Other series in Table 5 %
Burning pain	24	96	85-100
Itching	6	24	24
Swelling	24	96	65-94
Erythema	9	36	20-85
Erosions	3	12	14-15
Blisters	1	4	3-17
Petechia	1	4	3-15
Chronic changes	9	36	15-67
Face	4		
Hand	7		
Total number of patients	25		275

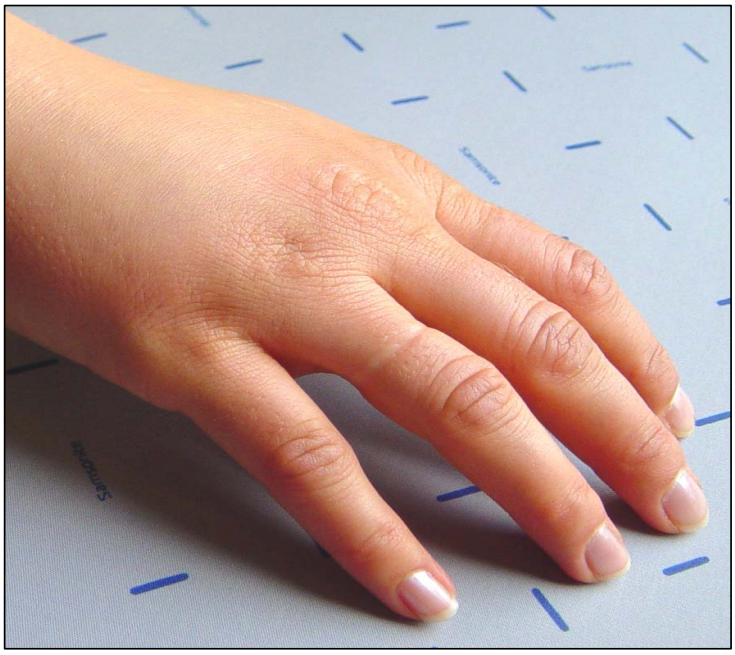


Figure 13. Acute swelling of the hand in a patient with EPP (on the courtesy of the patient)

Figure 14. Petechial photoreaction on the face skin of a patient with EPP (on the courtesy of the patient)

Profile of skin manifestations in Finnish patients with EPP did not differ from those reported in other series (de Leo et al. 1976; Lehmann et al. 1991; Holme et al. 2006a) and thus, our patients represented general features of the disease. The Finnish patients with EPP have had skin symptoms from three to six months during the year mainly due to the climatic light exposure.

The severity of the photosensitivity correlated to the erythrocyte protoporphyrin concentration (Article V, Figure 15). The patients with high erythrocyte protoporphyrin levels (> 50 000 nmol/l) experienced more severe photosensitivity than the patients with moderate levels. The severity and frequency of skin symptoms also varied according to the individual sun exposure and annual variation of the sunny weather.

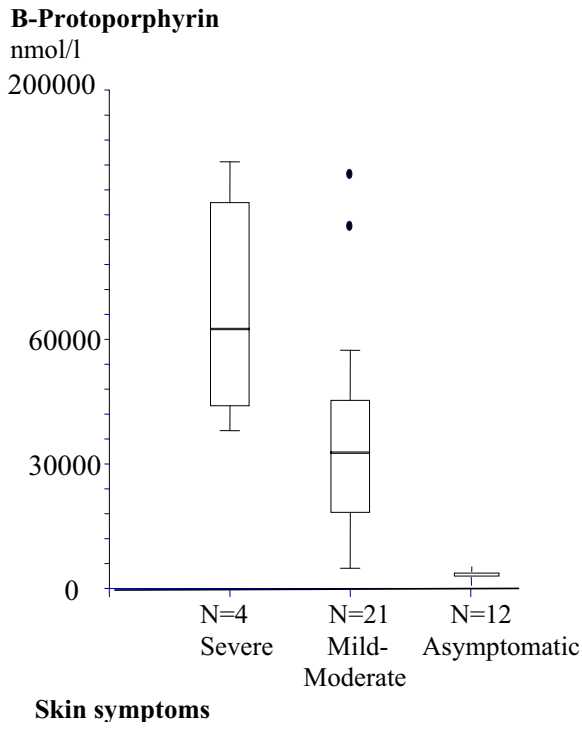


Figure 15. Blood protoporphyrin levels in subgroups of the patients with EPP.

3.2.2. Liver disease

Hepatopathy related to EPP occurred only in one of the 25 symptomatic patients. He was 20-years-old patient with moderate photosensitivity and high levels of erythrocyte protoporpnhyrin (mean 87 000 nmol/l). Since the age of 12 the serum transaminase levels have fluctuated up to 6-fold concomitantly with the increased level of erythrocyte protoporphyrin (up to 100 000 nmol/l). In liver biopsy increased pigmentation in hepatocytes and Kupfer cells could be demonstrated suggesting accumulation of protoporphyrin. Only mild inflammatory changes without cholestasis could be observed.

A temporary increase of serum transaminase levels occurred in an adult man with severe skin symptoms and constantly high level of erythrocyte protoporphyrin. In addition, a 13-year-old boy at his puberty experienced a slight and transient increase of serum alanine transaminase level and a simultaneous increase of the erythrocyte protoporphyrin level. In liver biopsy the histology and ultrastructure of the liver were normal. The majority of the patients with high erythrocyte protoporphyrin concentrations (>50 000 nmol/l) had normal liver function during the follow-up. Bile stones were operated in one female patient only.

Although the liver complications in EPP are well recognized, there are only few studies on

the frequency of liver disease. In our small series the occurrence of hepatopathy is comparable to that reported from the Netherlands, where 5–10% of EPP patients experienced liver disease (Baart de la Faille et al. 1991). So far, cases of liver failure have not occurred in Finland. Compared to Germany, where up to 30% of EPP patients have hepatopathy (Doss and Frank 1989), liver complications appear to be less common in Finland. The exact pathogenesis of the liver disease in EPP has not been clarified entirely. It has been hypothesized, that hepatic injury is caused by crystalline deposits of protoporphyrins in the hepatocytes and bile canaliculi accompanied by hepatobiliary obstruction (Bloomer et al. 2005). The development of a liver disease also may be influenced by inter-individual variation of hepatic production, clearance and excretion of porphyrins determined by genetic factors (de Verneuil et al. 1995). Further, protoporphyrin may have a toxic hepatocellular effect *via* generation of oxygen free radicals resulting in oxidative damage (Koningsberger et al. 1995). High levels of erythrocyte protoporphyrins may be associated with development of the liver disease but the values cannot be used to predict it.

Table 18. The results of the liver function tests and the haematological measurements in 37 EPP patients during the follow-up

	Clinical manifestations			
	No symptoms	Mild-moderate skin symptoms	Severe skin symptoms[3]	P-value
	N=12	N=21	N=4	
	Mean (range)	Mean (range)	Mean (range)	
B-Protoporphyrin, <1050 nmol/l	1668 (1000-3153)	36114 (4847-99369)	66453 (38000-102699)	0.000002[1] 0.03[2]
Liver tests	N=2			
AST, <50 U/l	41 (23-58)	30 (17-62)	33 (29-37)	
ALT, <50 U/l	38 (20-55)	29 (12-103)	32 (17-45)	
GGT, <50 U/l	n.a.	35 (10-153)	17 (14-19)	
Haematological tests				
	N=4			
Hb, 117-164g/l	136 (116-154)	128 (97-150)	131 (126-140)	
MCV, 80-95 fl	89 (83-97)	80 (65-87)	79 (78-79)	0.003[1]
MCH, 27-32 pg	30 (27-33)	26 (21-29)	27 (27-27)	
S-Ferritin, male 10-200 ug/l female 5-90 ug/l children > 6 ug/l	N=1 235	Male N=7[4] 31 (7-74) Female N=7 20 (13-37) Total 23 (7-74)	Male N=3 13 (12-14) Female N=1 7 Total 11 (7-14)	

n.a. not applicable,
[1]ANOVA used in comparison of the patient groups,
[2]Mann-Whitney test used to compare of the symptomatic patient groups,
[3]Severe: pain, oedema and/or erythema in less than 15 min after exposure to the sun
[4]One patient with increased serum ferritin value related to alcohol liver disease was excluded

3.2.3. Haematological findings in patients with EPP

The means of haemoglobin values were within the normal levels in the majority (77%) of the patients, although many of them were at the low level of normal range (Table 18). Serum ferritin levels were within the current normal range, but in 10 of the 18 patients (56%) the means were near the lower limit of normal (7-15 ug/l).

In the peripheral blood smear of, hypochromacy and microcytes were a constant finding in symptomatic patients and occurred in all eight cases examined. Ovalocytes were quite frequent (38%) resembling iron deficiency. In one of three asymptomatic patients a few microcytes were found, otherwise the red cell morphology was normal. In other series mild anaemia was found in 20-50% of the patients (de Leo et al. 1976; Went and Klasen 1984; Baart de la Faille et al. 1991), which is in accordance with our results.

3.3. Porphyria cutanea tarda

3.3.1. Skin symptoms

Of 85 patients with PCT 81 subjects experienced skin symptom. In three patients of them (one type I and two unclassified cases) no detailed clinical information was available but the diagnosis of PCT was confirmed in dermatological units. Forty five patients were examined clinically by the author. The information about 33 patients was collected from the patient records and consequently the data could be incomplete.

Table 19. Demographic data of patients with PCT[1]

Number of patients	Males	Females	Total	Mean age, y (range)
Type I	27	9	36	55 (28-71)
Type II	19	7	26	52 (30-73)
Type III	1	1	2	43 and 70
Unclassified	17	4	21	50 (25-66) [2]
Total number of patients	64	21	85	

[1]including asymptomatic patients (no UROD activity known)
[2]Two patients without information exluded

Men were more commonly affected compared to women (76 % to 24 %) in all subgroups of PCT. The number of patients having the sporadic type of PCT (Type I) was comparable to that of the familiar type (type II), but 25 % of the cases could not be classified because their UROD activities had not been measured. Only one family with normal UROD activity in erythrocytes was identified in Finland. The age of the onset of the skin symptoms varied substantially in all groups. In men the mean age was 52 (25 to 71) years and in women 55 (28 to 73) years respectively. No difference at the age of the onset was observed between the groups.

The major skin manifestations in the patients with PCT were the blistering (95%) and skin fragility (93%) on the back of the fingers and the hands (Table 20). Most of the blisters were small (=<1cm) in size, but in 44 % of the patients bullous lesions also (blisters exceeding 1 cm up to 4-5 cm in diameter) were present. All patients, except two, who experienced skin fragility on the back of the hands, had also blistering lesions. One patient with blistering lesions on the back of the hands reported no skin fragility. In four patients who had blistering lesions, the presence or the absence of skin fragility was not recorded and respectively, in one patient with

skin fragility the information about blisters was lacking. One third of the patients experienced skin fragility on the face and 36% (10/27) of them had blistering lesions too. The minority of the patients experienced skin fragility and/or blistering in other sun-exposed areas including arms, legs and feet (Table 20). The erosions and blisters left initially dark red scars which could commonly be found on the back of the hands even in absence of erosions. Red colour faded gradually within months and could disappear completely during remission. The milia formation on the scars was quite common.

Table 20. Characteristics of skin symptoms in PCT

	Number of symptomatic patients						Other series[1]
Symptom	T I	T II	T III	Unclassified	Total	%	%
Erosions (fragility)	31	22	1	15	69	93[2]	
Hand	31	22	1	15	69	93	93
Arm	5	4	-	2	11	15	
Face	15	6	-	6	27	36	62
Foot /leg	2	3	-	-	5	7	
Blisters in the hand skin	33	22	1	17	73	95[3]	93
Bullae	14	12	-	8	34	44	
Scars	31	22	1	14	68	87	
Milia	13	9	1	7	30	38	36
Sclerodermoid changes	1	-	-	1	2	3	4
Hypertrichosis	18	9	-	4	31	70[4]	56
Hyperpigmentation	2	5	-	4	11	24[5]	33
Total number of patients	34	24	1	19	78		

[1]Bygum et al. (2003) [2]Four patients without information about skin fragility and [3]one patient without information about blisters were excluded from the percentages
[4,5]Calculated on the bases of [4]44/46[5] patients with information

The data about hypertrichosis and hyperpigmentaton were documented in less than 60% of the patients and thus, the results may be biased. Hypertrichosis was noted in two thirds of the cases (Table 20). The location was most frequently periorbital areas preferably on the top of the cheeks but also other malar areas, and in women, the chin was affected. Hypertrichosis varied from lanugo to long, dark and thick hairs. The presence of hyperpigmentation on the face was noted in one fourth of the cases (Table 20), of which six were women. It occurred as brownish pigmented blotches on the temporal and malar areas or it was evenly distributed over the whole face skin like a dark tanning. Of note, in one patient hyperpigmentation was the only skin manifestation of PCT. Another patient also had hypertrichosis in addition to hyperpigmentation, but no skin fragility. Both of them were hospitalized because of other diseases and thus, they were not exposed to the sunlight, which would explain the absence of phototoxic reactions.

The frequency of blistering and fragility of the hand skin was similar in the subgroups of PCT (Table 20). The presence of hyperpigmentation was more common in type II than type I PCT (5/17 and 2/21 respectively), while in the frequency of hypertrichosis was no signicant difference between types I and II PCT (18/23 and 9/15 respectively)**.** The clinical manifestations of the Finnish patients with PCT did not differ from the patients in other series (Grossman et al.

1979; Bygum et al. 2003). Compared to VP the blisters were present nearly in all patients with PCT, but in less than half of the patients with VP.

Two patients with photosensitivity were excluded from our series since their skin lesions were atypical despite the typical porphyrin pattern in their plasma and porphyrin excretion in urine. A 66-year-old man who had no typical photosensitivity of PCT experienced polymorphous light eruption and eczema-like skin lesions. The profile of urinary excretion of porphyrins was typical for PCT (60-fold increase of uroporphyrin, increased 7-carboxyl porphyrin, and 2-fold increase of coproporphyrin) with normal faecal porphyrin excretion. The other patient, who was a 48-year-old man with polymorphous light eruption, had an increased excretion of urinary uroporphyrin (40-fold) and 7-carboxyl porphyrin (139-fold), plasma porphyrin emission peak at 617 and normal faecal porhyrin excretion. Because lichenoid scars on the back of the hands were noted, it was possible that he also experienced fragility or blistering of the skin which were ignored. These patients did not co-operate for treatment of PCT or for further investigations such as liver ultrasound in order to exclude liver tumours. The association of abnormal porphyrin pattern and their skin lesion is uncertain and may have been found only by chance in plasma and urine analysis.

3.3.2. Liver disease

The serum transaminase levels were increased in the majority of patients examined (Table 21) as in previous series (Sarkany 2001). The levels of alanine transaminase and gamma- glutamyl transferase were more commonly increased than that of aspartate transaminase. The mean levels of transaminases were increased in greater degree in type II PCT than in type I, or in the unclassified group or in a single case of type III. However, great variation of values existed in all subgroups of PCT. The results indicate that the patients with type I and II are similarly affected by a liver disease. Liver imaging, mostly by ultrasound was performed in 80 % of the patients (Table 22). Abnormal findings occurred approximately in half of the patients and their frequency was similar in type I and II groups (64% vs. 52%). The most common abnormality was fatty degeneration (48%). No hepatoma was found, but one patient had metastasis in the liver, and thus, imaging should always be performed to look for paraneoplastic etiology of increased porphyrin excretion.

Table 21. The results of the liver function and iron metabolism tests in patients with PCT

Biochemical analyses, normal range	Type I Mean (range) N=32	Type II Mean (range) N=22	Type III (Mean) N=1	Unclassified Mean (range) N=18	All patients Mean (range) N=73	Abnormal values %
AST, <50 U/l	60 (28-148)	78 (28-211)	21	53 (27-95)	64 (21-211)	57
ALT, <50 U/l	82 (22-186)	111 (16-283)	26	88 (30-173)	91 (16-283)	82
GGT, <50 U/l	97 (16-495)	120 (28-318)	65	104 (28-268)	105 (16-495)	78
S-Ferritin, male 10-200 µg/l female 5-90 µg/l	538 (71-1864)	795 (158-3770)	364	544 (234-1697)	634 (71-3770)	93
S-Transferrin saturation, 17-52%	48 (24-87)	51 (33-77)	39	46 (27-89)	47 (24-89)	38
Hb, 117-164 g/l	152 (107-182)	158 (138-185)	142	153 (136-173)	154 (107-185)	
MCV, 80-95 fl	95 (88-106)	94 (84-104)	106	91 (80-104)	95 (80-106)	50

Table 22. Results of liver imaging in patients with PCT						
	Type I	Type II	Type III	Unclassified	Total	%
Ultrasound, scintigraphy, computed tomography,	N=28	N=21	N=1	N=13	N=63	
Finding						
I. Normal	10	10	1	6	27	43
II: Abnormal	18	11		7	36	57
Fatty degeneration	13	10		7	30	48
Gall stones	1	1			2	3
Metastasis	1				1	2
Peliosis	1				1	2
Cysts	2	2			4	6

Liver biopsy was carried out in 21 patients (Table 23). Histological changes were observed in all samples studied as in previous series of 16 patients (Campo et al. 1990). The most common finding was siderosis (67%) and fatty degeneration (67%) in our series. Fibrosis suggesting a cirrhotic stage of the liver damage was present in 14 % of the patients. Porphyrin crystals (Cortes et al. 1980) were identified in two samples. Periportal deposits of haemosiderin in hepatocytes may be a more specific sign of hepatopathy related to PCT (Campo et al. 1990) especially when associated with fatty changes and portal inflammatory process.

Table 23. Results of the liver biopsy in patients with PCT						
	Type I	Type II	Type III	Unclassified	Total	%
Liver biopsy	N=7	N=7		N=7	N=21	
I. Normal						
II. Abnormal	7	7		7	21	
Fatty degeneration	2	7		5	14	67
Inflammation	2	1			3	14
Siderosis	3	5		6	14	67
Fluorescence				1	1	5
Peliosis	1				1	5
Fibrosis	2	1			3	4
Lymphoma	1				1	5

3.3.3. Associated factors

The level of serum ferritin were increased in the majority (93%) of the patients with PCT (Table 21) as reported elsewhere (Bulaj et al. 2000). The mean level of serum ferritin was 3-fold compared to the normal range. Around half of the Finnish patients with PCT have at least one of the mutations associated with hemochromatosis. The frequency of the C282Y mutation in our patients (30%) was increased 3-fold compared to that of the normal Finnish population (10%), while the frequency of the H63D mutation (20 %) was similar as estimated in the normal Finnish population (Parkkila et al. 2001). The frequencies of homozygous C282Y mutation (9%) and compound heterozygous C282Y/H63D mutations (6%) among patients exceeded distinctly those estimated in the normal Finnish population (0,4% and 2 % respectively) (Parkkila et al. 2001). These results are in accordance with the results of other series although our figures are lower

than the figures presented from US or Sweden (Bulaj et al. 2000; Egger et al. 2002; Harper et al. 2004). The results support the hypothesis that liver siderosis in PCT associates with *HFE* mutations in the significant number of patients. Iron metabolism including the *HFE* genotyping should be investigated in the patients with PCT in order to reveal potential hemochromatosis in these patients.

A low frequency of viral hepatitis among Finnish patients with PCT could explain the low occurrence of PCT. In other countries, where the prevalence of HCV infection is also low, e.g. in the United Kingdom (Elder 1998) and Germany (Stolzel et al. 1995), the frequency of PCT is significantly higher than in Finland. The chronic hepatitis B and C infections are more common in Mediterranean countries (Rocchi et al. 1986; Stransky et al. 2000) than in the northern part of Europe, and this may partly explain high prevalence of PCT in the southern part of Europe.

The use of oestrogen preparations for contraception and postmenopausal replacement therapy is in Finland as widespread as in other western countries (Topo et al. 1991). Thus, the known risk factors poorly explain the low prevalence of PCT in Finland. A specific gene defect linked to a deficient liver UROD in addition to the associating factors is probably required for manifestation of the disease. At present the major triggering factors for manifestation of PCT in Finnish patients with PCT is the use of alcohol and siderosis, and oestrogen therapy. Despite heavy use of alcohol is customary in Finland (Helasoja et al. 2007), PCT is a rare disease in our country.

Table 24. Associated factors in different subgroups of patients with PCT

	T I	T II	T III	Unclassified		
	Number of patients with information in each group /positive finding				Total number of patients/positive finding	
						%
Alcohol intake increased	N=30/23	N=23/16	N=1/1	N=17/15	71/55	78
Number of females/use of oestrogen preparations	N=9/2	N=7/2		N=4/1	20/5	25
	Number of patients studied/positive finding					
Viral hepatitis						
A	N=9/2	N=6/3	N=1/0	N=4/0	20/5	25
B	N=28/5	N=17/0	N=1/0	N=3/0	47/5	11
C	N=23/3	N=17/2	N=1/0	N=1/1	42/6	14
Hemochromatosis diagnosed clinically	N=3	N=1	N=0	N=1	5	
C282Y mutation						
Homozygotes	N=13/3	N=18/0	N=1/0	N=1/0	33/3	9
Heterozygotes	N=13/4	N=18/3	N=1/0	N=1/0	33/7	21
H63D mutation						
Homozygotes	N=14/0	N=18/0	N=1/0	N=1/0	34/0	0
Heterozygotes	N=14/1	N=18/5	N=1/0	N=1/1	34/6	18
C282Y/H63D compound heterozygotes	N=1	N=2	N=0	N=0	33/2	6

4. THE HISTOPATHOLOGY OF THE SKIN (Articles I, V, VI)

4.1. Variegate porphyria

4.1.1. Subjects

When the histological study was performed in 1989 no DNA analysis of PPOX gene was done and mutations were identified only later (Table 25) (von und zu Fraunberg et al. 2002; von und zu Fraunberg et al. 2005). The numbers in each group were small except in the group of the major mutation (C454T) found in Finland, and thus, no comparison between genotype and histological findings in the groups could be performed. In Group III only parents with a homozygous child (Article III) were included because they were obligatory gene carriers and later on their mutation was characterised. It was hard to find such patients in other families and only later on more patients were characterised with mutation screening.

Table 25. Mutations identified in the patients with VP in the groups I-III

Mutation	C454T	G338C	T35C	A470C
N= 20, patients				
Group I, current skin symptoms, mean age 41 years	10	1	0	1
Group II, no skin symptoms but increased porphyrin excretions, mean age 50 years	5	0	1	0
Group III, no skin symptoms and normal porphyrin analyses, mean age 36 years	0	0	2	0
Total number of patients	15	1	3	1
Controls N=5, mean age 45 years				

4.1.1. Light microscopy

Patients with VP were grouped into three categories based on their skin symptoms and biochemical findings (Tables 1 and 3, I). The histopathological, immunohistological and ultrastructural changes of the skin were evaluated in each group (Tables 3 and 4, I). In all patients with the skin fragility (Group I, I) the principal finding of the previously affected unbroken skin was thickening of the superficial dermal vessel walls. The change was best demonstrated in PAS-staining (Figure 1, I). Mild thickening of the epidermal basement membrane was only occasionally observed in symptomatic patients.

In the non-exposed skin mild thickening of the vessel walls could be demonstrated in the majority (70%, 8/12) of the patients. In the others, the vessels were normal. The sun-exposed skin of the symptom-free patients with an increased faecal protoporphyrin excretion (Group II, I) showed thickening of the vessel walls in 80 % (5/6) of the patients. In the symptom-free family members with normal porphyrin excretions (Group III, I and III), the vessels were normal. In the epidermal basement membrane zone pronounced PAS-positive findings were detected in two thirds (8/12) of symptomatic patients, but in none of asymptomatic individuals.

4.1.2. Electron microscopy

Electron microscopic examination demonstrated that thickening of the vessel walls was caused by multilayering of the basal lamina and deposit of an amorphous material around a vessel (Fig 2a-c, I). In the affected skin (Group I) thickness of the vessel walls was 2-3 fold compared to that of normal (Braverman and Keh-Yen 1984) and the changes were graded as moderate or severe (>5μm) in the majority (80%, 10/12 patients) of the cases (Table 3, I). The basal lamina consisted of 5-10 layers usually, but in most extensively altered vessels up to 20 layers. Electron dense amorphous material was observed as a broad belt around the basal lamina zone. Thin collagen fibres were found in this material. Perivascular dendrocytes (veil cells) surrounded the excess material and separated vessels from dermal collagen. Endothelial cells and pericytes were intact in the vessels studied.

In the non-exposed skin the vessel walls showed thickening, which was overall slight, in 60% (7/12) of the patients. In the others the vessels were classified as normal. Thus, the ultrastructural findings were in accordance with those observed by LM. The morphology of the thickened vessel walls resembled that of the sun-exposed skin, but scanty perivascular material was demonstrated only in two patients.

In the group II the vessel walls exhibited thickening graded moderate or severe, in 70 % (4/6) of the cases (Table 3, I) in accordance with the findings of the LM. The reduplication of the basal lamina was less marked in this group compared to the affected skin of the symptomatic patients suggesting that basal lamina was spared from serous injury. Amorphous extra material was present, but not so abundantly in comparison to the symptomatic patients.

The blister of exposed skin studied in three patients located under the lamina densa of the epidermal basement membrane. In the blistered area basal keratinocytes were normal and basal lamina was intact with normal haemidesmosomes or it was discontinuous. The basal lamina seemed to rupture when the substantial amount of blister liquid accumulated under the epidermis.

4.1.3. Direct immunofluorescence

DIF revealed homogenous IgG deposits constantly in the vessel walls of the sun-exposed skin in the symptomatic patients (Group I) (Table 4, I). IgA and IgM deposits could be demonstrated less commonly (7/12 and 5/12 respectively) and C3 was seldom present. IgG was identified in the vessel walls only in one fourth of the patients in the sun-protected skin. IgG deposits in the vessel walls could also be demonstrated in the samples of four symptom-free patients studied (Group II). In the epidermal basement membrane IgG was demonstrated in half of the symptomatic patients, but also in a few symptom-free patients with an increased excretion of faecal protoporphyrin.

4.1.4 Discussion

According to our series of 12 symptomatic patients with VP and nine additional patients with skin symptoms reported by other groups (Rimington et al. 1967; Epstein et al. 1973; Corey et al. 1980; Westerhof and Smit 1981; Maynard and Peters 1992; Grabczynska et al. 1996), the major histopathological abnormality of the affected skin is the thickening of superficial dermal vessel walls in the dorsal side of the hand which suggests that the vessel walls are the most susceptible to photodamage in VP. The electron microscopic examination revealed that the principally injured region is the basal lamina zone. The most prominent changes could be demonstrated in venules, which are physiologically the most reactive part of microcirculation in the skin and thus, more vulnerable than arterioles (Braverman and Keh-Yen 1984). In the sun-exposed skin of a sole patient with VP studied previously by EM, the basal lamina of the vascular basement membrane was as extensively reduplicated as in our patients (Epstein et al. 1973). The excess material within and beyond the widened perivascular spaces was described as finely fibrillar in samples of this subject, but irregular clumps of dense amorphous material also were noted in perivascular deposits (Epstein et al. 1973). In our samples the excess material had a more amorphous appearance and it was ranged regularly outside the basal laminae.

The results of DIF in the sun-exposed skin of 12 patients with VP were comparable to the six patients described previously (Epstein et al. 1973; Corey et al. 1980; Maynard and Peters 1992; Grabczynska et al. 1996). The common finding has been IgG and/or IgM deposits in the vessel walls of the upper dermis in the sun-exposed skin. Complement deposits in the vessel walls were found infrequently in our patients' samples which is in agreement with a few cases reported previously (Epstein et al. 1973). The invasion of immunoglobulins in the skin is probably only a secondary phenomenon due to the damage of the vessel walls. The lack of inflammatory cells also in the skin samples taken during blistering phase, suggests that the presence of immunoglobulins originates from serum. The less common presence of IgA and IgM compared to IgG is probably due to their larger molecular size hindering the migration outside the lumen. Other authors have also proposed that deposition of immunoglobulins in the vessel walls is rather derived from circulation than related to immune response based on the lack of circulating antibodies and an irregular attendance of complement (Epstein et al. 1973). Thus, our and the earlier results do not directly support the hypothesis of the activation of the complement cascade by porphyrin induced phototoxic reaction as presented in PCT and EPP (Gigli et al. 1980; Lim 1989).

According to our results, the morphological changes detected in the vessel walls were chronic, because they were present also in old lesions without coincident fragility. The vascular changes could also be demonstrated in those skin samples which were taken during blistering and eroding phase showing that vascular changes were present in the early phase of skin damage. However, it is still obscure, how vascular changes are related to the skin fragility and blistering. The blister location under the lamina densa of the epidermal basement membrane suggests that the blister formation arises from the dermal side. Photodamage may be initiated in the vessel walls via interaction of circulating porphyrins and solar radiation (Poh-Fitzpatrick 1985) but no experimental studies have been performed in VP to clarify this hypothesis. Thus, the exact mechanism of the damage in the epidermal basement membrane zone is still unclear. Studies with the long-term follow-up including repeated skin biopsies would elucidate more precisely the natural course of microscopic alterations but they have not been reported so far.

In our series the changes of the sun-exposed skin were definitely more prominent than those of the non-exposed skin indicating that sun exposition is crucial in development of microscopic changes in the skin. Because the basal lamina of the normal venule wall is multilayered consisting usually of 2-4 layers (Braverman and Keh-Yen 1984), the interpretation of the mild

reduplication as an abnormal finding should be done with caution.

Based on our results the slight changes detected in the light and EM suggest that photoactivated porphyrins may cause changes in the non-exposed skin via circulation or an excess of porphyrins *per se* without photoactivation may be able to elicit vascular changes. However, even minor solar exposure at the presence of porphyrins could be sufficient to induce microscopic alterations which could explain the minor changes in the non-exposed skin. Uninvolved skin has been studied histologically earlier in a single patient with VP (Epstein et al. 1973). The arm skin, which may be more exposed to the light than the gluteal skin used in our series, showed no histological changes.

In the non-exposed skin immunoglobulins in the vessel walls could be demonstrated only in occasional cases suggesting that vascular damage was too mild for leakage of immunoglobulins. In an earlier study IgG could be demonstrated in the vessel walls of the uninvolved arm skin in two patients with VP although the morphology of the vessel walls was normal (Epstein et al. 1973). This could be an unspecific phenomenon or a minor damage related to photoactivated protoporphyrins.

The microscopic and immunofluorescent changes in the vessels of the sun-exposed skin in the symptom-free patients with abnormal porphyrin excretions (Group II) resembled considerably those of symptomatic patients indicating also the vascular damage in this group. The regular presence of homogenous immunoglobulin deposits in the vessel walls supports the hypothesis that the vascular changes are related to phototoxic injury resulting in the outflow of immunoglobulins. Although this group was small the histopathological finding suggested that characteristic vascular alterations could develop without clinical symptoms. Thus, photodamage at the level of dermal vessels may be a subclinical process in VP and does not occur concomitantly with clinical symptoms. During the follow-up of twenty years after the skin biopsy none of our patients experienced skin fragility indicating that microscopic alterations have no prognostic significance for the occurrence of the skin manifestations later.

Since photoageing of the skin may cause similar thickening of the vessel walls as found in porphyria (Kumakiri et al. 1977; Braverman and Keh-Yen 1984), the relationship of vascular changes to porphyria is not unambiguous considering the mean age of 50 years in group II. Moreover, in our control patients aged over 50 years, amorphous extra material could be demonstrated in a few vessel walls (Article I). The symptom-free family members with normal porphyrin excretion and normal vessels were substantially younger (36 years old) than those with abnormal prophyrin metabolism. Thus, ageing could partly explain the histological changes in group II. Since the mean age of symptomatic patients (Group I) was 41 years and the extent of vascular alterations was massive, the photoageing unlikely was of any significance in these patients.

It is still an open question why only 30% of the patients have skin symptoms despite increased porphyrin level in the circulation. A specific mutation in the PPOX gene affected the phenotype of the disease to some extent. For example the parents of the homozygous patient, who formed group III in this study, had no histological changes and none of their heterozygous relatives had skin symptoms during the follow-up (Article III; von und zu Fraunberg et al. 2002). The I12T mutation has a residual activity of 8% when measured in COS-1 cells (Article III), which may associate with a lack of skin symptoms at the heterozygous state or the family members may have inherited concomitantly currently unknown protecting agent. Of note, the major mutation (C454T) has also a residual activity of 5% when measured in vitro conditions (von und zu Fraunberg et al. 2001) and excretion of porphyrins is comparable to that of G338C with no residual activity indicating that the severity of histological changes does not solely depend on the mutation in the PPOX gene but other factors such as sun exposure and modifying genes are involved and may play a major role in the presence of a mutation.

Excretion of porphyrins especially urinary coproporphyrin and faecal porphyrins in the different subgroups was the most important predicting number (Article I) and this could be seen also in the larger series 10 years later (von und zu Fraunberg et al. 2002). The occurrence of skin symptoms may be related to individual differences in acceleration of porphyrin synthesis in the liver and their elimination from the circulation. Modifying genes related to the function of cytochrome P450 enzymes may influence regulation of porphyrin metabolism in the liver (von und zu Fraunberg et al. 2005). In addition, the removal of porphyrins from the skin or the local porphyrin production in the skin may vary individually (Day 1986). The level of plasma porphyrin was not measured in our study and their correlation to the skin symptoms is not known. The main location of skin manifestations in the back of the hands and on the face suggests that skin symptoms are related only to the chronic sun exposure. The more frequent localization of skin symptoms on the hands compared to the face indicates that mechanical trauma aggravates skin damage in consequence of foregoing or concomitant phototoxic reaction.

4.2. Erythropoietic protoporphyria

4.2.1.Subjects

After the histological study was done, the mutation analysis was performed in four families (Table 26). In three cases the patients were the only symptomatic patient in the family, and thus, comparison of the histological findings between different mutations was not done but the results were pooled. The age of the patients varied from 7 to 43 years and the gender distribution was similar (Table I, V). Duration of the symptoms varied considerably 3-43 years (Table I, V).

Table 26. Mutations, clinical and histological findings in patients with EPP[1]

Mutation	Patients studied/ symptomatic patients in the family	B-Protoporphyrins mean value during the follow-up 1988-1999 (normal range 200-1010 nmol/l)	Severity of photosensitivity	Severity of LM changes	Severity of EM changes
C286T	1/1	31316	++	++	++
C343T	1/2	78785	+++	+++	+++
751GAGAA	2/3	48916	+++	++	+++
		47444	++(+++)	++	+++
Exon 10del1122T	1/1	12207	+	++	++
Mutation unknown	1/1	27454	++	+++	ND
	1/3	55925	++	+++	++
	1/3	71734	+++	+	ND
	8/14				

[1](IV) and (V)

4.2.2. Light microscopy

In LM of the sun-exposed skin of EPP patients the most striking changes were massive PAS-positive homogenous deposits around the vessels and thickening of the superficial vessel walls (Fig 1, V). The deposits located both in the close vicinity to basement membrane zone and separately from vessels (Fig 1, V). The changes were present distinctly in the superficial part of papillary dermis also. The severity of the histological changes did not clearly correlate with the age of a patient or duration of the skin symptoms in this series (Table I, V). Moreover, the levels of erythrocyte protoporphyrin did not predict the severity of the microscopic changes. The vessel walls were normal in thickness in the non-exposed skin and no abnormal deposits could be identified.

4.2.3. Electron microscopy

In the EM, thickening of the vessel walls was caused by concentric reduplication of basal lamina and excess of the fine granular and homogenous material between the layers of basal lamina and around the basal membrane zone (Fig 2, V). The thickness of the vessel walls varied from 3 to 8 μm. The deposits appeared as broad bands in the perivascular space and as large clumps with no direct contact with the vessel walls (Fig 3, V). The clumps were more homogenous and electron dense than deposits in the vessel walls. Fibroblasts and their projections surrounded these masses. The morphology of fibroblasts and collagen bundles near the deposits was normal. Mast cells occurred close to the vessels in both sun-exposed and non-exposed samples. Endothelial cells were normal indicating that the damage caused by the solar exposure is an acute and transient phenomenon.

4.2.4. Direct immunofluorescence

DIF examination demonstrated that IgG deposits in the vessel walls were a constant finding (Table II, V). In addition, a weak or moderate IgA and IgM fluorescence was found in the majority of cases (7/8 and 6/8 respectively), and complement in half of cases (4/8).

4.2.5. Immunohistochemistry

The immunohistochemical study confirmed that the thickened vessel walls and amorphous material around them composed of the excess of the basement membrane materials and proteins derived from the circulation. Collagen IV and laminin, which are normal constituents of the vascular basement membrane, were most extensively expressed (Fig 4-5, V). Collagen IV was expressed in the vessel walls and in the depositions outside the vessels, while laminin was confined in the vessel walls. In addition, constituents derived from the circulation including serum amyloid protein (SAP) and light chains kappa and lambda were demonstrated in the amorphous material around the vessels (Figs 6-8, V). Light chains kappa and lambda were identified in the vessel walls and in trace quantity in the surrounding material. The 90 kD glycoprotein associated with hyalinosis could be sparsely demonstrated in the amorphous material (Fig 8, V)

4.2.6. Discussion

The major abnormalities of the sun-exposed skin in our eight EPP patients included the extensive reduplication of basal lamina and homogenous deposits around the vascular basal membrane zone indicating that the main damage in EPP arises in the upper dermal vessels, which are affected by interaction of circulating protoporphyrin and solar radiation (Poh-Fitzpatrick 1985). These findings are in accordance with the previous studies in which PAS-positive amorphous material was demonstrated in and around vessel walls of the upper dermis of the sun-exposed skin of EPP patients (Peterka et al. 1965; Findlay et al. 1966; Ryan 1966; Anton-Lambrecht and Bersch 1971; Epstein et al. 1973; Baart de la Faile 1975). Of note, the microscopical changes were demonstrated in our series during asymptomatic phase in normal looking skin suggesting that the changes are chronic. Based on our results the level of erythrocyte protoporphyrin showed no clear correlation with the severity of the microscopical findings.

In the non-exposed skin, the vessel walls were normal in thickness and no abnormal deposits could be identified in accordance with an earlier histopathological study including eight patients with EPP (Rimington et al. 1967). These findings indicate that the solar exposure is mandatory for the development of the vascular changes and probably the most important factor in the variance of histopathological findings in patients' samples. The target for the phototoxic damage is probably an endothelial cell resulting in widening of the intercellular gaps and a leakage of circulating material from the lumen to the extracellular space as demonstrated earlier (Gschnait et al. 1975). Since the sun-protected skin was normal, the excess of free circulating protoporphyrin is non-toxic to endothelial cells *per se*, but solar exposition is needed for the damage. In our study, which was carried out at the asymptomatic phase, the endothelial cells were intact and no gaps between them were found indicating that the endothelial damage is an acute and reversible phenomenon. Because mast cells were present both in the sun-exposed and non-exposed samples, the results did not clarify the role of mast cells, which had been connected previously to acute phototoxic reaction (Glover et al. 1990).

The presence of PAS-positive material in the vessel walls and deposits suggests the accumulation of neutral mucopolysaccharides or glycoproteins, but the precise constitution of the deposits has been poorly characterised. Our results revealed that the material in the affected vessel walls and deposits contains various serum originating proteins such as immunoglobulins, complement and kappa and lambda light chains and serum amyloid protein (SAP) in addition to the normal basal membrane components. The more frequent occurrence of IgA and IgM in EPP compared to VP may be due to a more severe damage in the vascular wall in EPP allowing migration of larger proteins. The normal amount of immunoglobulins in the serum, a lack of inflammatory cells in the dermis and irregular occurrence of C3 in the vessel walls identified in our patients suggest that an immunological reaction is unlikely to be responsible for the accumulation of immunoglobulins in the vessel walls. This is more likely a result from a secondary leakage of the immunoreactants from the circulation. In the earlier study including 12 EPP patients, IgG has been demonstrated as a homogenous pattern in the vessel walls similar to our results (Epstein et al. 1973; Baart de la Faile 1975). In contrast to our study, IgG was present also in the epidermal basement zone.

Elastic fibre components e.g., SAP, fibronectin and vitronectin have been demonstrated in the peripheral part of perivascular deposit in the sun-exposed skin of EPP patients (Breathnach et al. 1983; Dahlbäck and Sakai 1990). Of the basal membrane components, accumulation of collagen type IV and laminin has been identified earlier in the affected vessel walls in two patients with EPP and four protoporphyric mice (Wick et al. 1979). The basal membrane glycoproteins are

most likely synthesised locally by fibroblasts, endothelial cells and pericytes during the multiple repairing processes after the phototoxic damage in the vessel wall**.** Our results and those reported by other groups (Ryan 1966; Epstein et al. 1973) indicate that the vascular thickening with perivascular deposits in EPP is secondary and irreversible phenomena resulting from the acute leakage and accumulation of different serum components and from the local production of basal membrane components. The 90 kD glycoprotein associating with hyalinosis could be sparsely demonstrated in the amorphous material (Fig 8, V) suggesting that the dermal deposits in EPP have different pathogenesis compared to cutaneous hyalinosis (Maury and Teppo 1984). Furthermore, absence of versican indicates that the acute phototoxic reaction found in EPP differs from that of the photoaged skin, in which versican is commonly expressed (Hasegawa et al. 2007).

4.3. Porphyria cutanea tarda

4.3.1 Subjects

Five patients with PCT who had experienced characteristic blistering and fragility of the sun-exposed skin from three months to two years before the diagnosis were subjected to repeated histopathological studies (Table 27). The erythrocyte UROD activity was normal in each of them suggesting type I of PCT. The urinary uroporphyrin excretion was increased 80-200-fold corresponding to moderate to severe increase of uroporphyrin excretion and responded well to choloroquine treatment (250 mg twice a week) within 6-17 months. Plasma porphyrin spectrum, showing fluorescence maximum at 617 nm, was measured initially only in one patient. It was later found in two additional patients during a relapse.

Table 27. Biochemical results and clinical information about five patients with PCT

Patients	Sex and age at the onset of photosensitivity	dU-Uroporphyrin at the time of biopsy normal <36 nmol/24h	E-UROD normal >60 pmol Copro-porph /h/mg prot	S-Ferritin normal male 10-200 ug/l female 5-90 ug/l	Provocating factors
1	M, 36 y	7890	69	1120	C282Y +/+ and hemochromatosis alcohol[1]
2	M, 63 y	3697	98	ND	alcohol
3	F, 62 y	4681	68	148	C282Y +/+[1]
4	F, 29 y	4280	72	ND	Hepatitis B and alcohol
5	M, 62 y	2778	60	158	alcohol

[1]After the study the patient was treated with phlebotomies

Skin biopsies were taken at the time of diagnosis of PCT in all patients, and as soon as biochemical remission (dU-Uroporphyrin ≤100 nmol/24h) was achieved in three patients, and after remission had lasted 6-12 months in all five patients**.**

4.3.2. Light microscopy

A principal finding in PCT on the light microscopic examination was a PAS-positive thickening of the superficial dermal vessel walls of the back of the hand (affected skin) (Fig 1, VI). In pretreatment phase the thickness of vessel walls was estimated to be moderate or severe in three out of five patients (Table 27, patients 1,2 and 5) and slight in the others (Table 27, patients 3 and 4). Comparison of histological findings in 2-3 consecutive samples of each patient demonstrated that vascular changes were not improved during remission except one case in which a severe vascular thickening was reduced to a slight degree (Fig 1, VI). In the non-exposed skin (gluteal area) thickness of the vessel walls was within the normal range before the treatment and during remission in three patients studied. The epidermal basement membrane showed no significant abnormalities.

4.3.3. Electron microscopy

The electron microscopic examination performed in the pretreatment phase displayed an abnormal reduplication of the basal lamina with 5-10 layers in the upper dermal vessels of the affected skin (Fig 2, VI, Table 27, Patient 1). The laminae were partially disrupted, but a concentric figure remained. The fine granular material was present between the layers. The basal lamina zone was surrounded by a broad belt of more amorphous material, which also contained a fine-grained substance. Perivascular dendrocytes (veil cells) surrounded this material and separated the vessels from dermal collagen. The thickness of vessel walls was calculated to be 5-8 μm in three, and 3-4 μm in one out of four patients examined (Fig 5, VI). Comparable morphological changes were demonstrated in the samples taken during remission in all patients (Figs 3-4, VI). At the end of the follow-up, the thickness of vessel walls was slightly reduced only in two cases, but exceeded 5 μm in all patients. Thus, no significant improvement was noted during remission. In the sun-protected skin the vessels showed a slight thickening throughout the study in one of three patients examined, and they were normal in the two others.

The blisters of the exposed skin studied in two patients in the pretreatment phase located under the lamina densa, which could not been demonstrated at the blister roof. A reduplication of the lamina densa close to blister was observed in the one of them. In other patients the epidermal basement membrane zone was normal.

4.3.4. Direct immunofluorescence

DIF examination demonstrated that before treatment a homogenous deposit of IgG was present in the vessel walls in the back of the hand in three of four patients examined. At the beginning of remission IgG was still expressed in the samples of the same patients**,** but at the end of the follow-up it was present only in one patient's sample. A prominent IgG deposit could be detected after one year remission in the fourth patient whose sample was negative for IgG before treatment. A slight deposit of IgA was demonstrated in one patient before treatment was still present at the beginning of remission but not at the end of the follow-up. In another patient a slight deposit of IgA was present first time at the beginning of remission and still after six months remission. IgM staining was negative in the vessel walls of all samples. In the sun-protected (gluteal) skin deposit of IgG was present in one patient in all consecutive samples. No immunoreactants could be demonstrated in the sun-protected skin throughout the study in the other patients' samples.

4.3.5. Discussion

In our series of five subjects who represent clinically and biochemically typical patients with PCT, the most important histopathological findings are thickening of the vessel walls in the back of the hands and formation of subepidermal blister. Our results correspond well with the previous histopathological studies describing skin changes in patients with PCT (Epstein et al. 1973; Kemmer et al. 1988; Maynard and Peters 1992; Vasil and Magro 2007). A few immunohistochemical studies, in which laminin, SAP and vitronectin have been demonstrated in the thickened vessel walls or in their vicinity, have been done to clarify the composition of the thickened vessel wall in PCT (Breathnach et al. 1983; Dahlbäck and Sakai 1990). Accumulation of collagen IV has been revealed in the thickened vessel wall in one patient with PCT (Krajnc et al. 1998). These findings could indicate that the components are present because of active scarring process but also because of the leakage from the circulation. The blisters studied in a few patients with PCT had a subepidermal location, under the basal lamina.

Electron microscopic investigation demonstrated that the thickening of the vessel walls was related to multilayering of the basal lamina and accumulation of amorphous material at the basal membrane zone in our series. Reduplication of vascular basement membrane and excess of fine fibrillar or amorphous material between and outside the reduplicated basal laminae have been identified also in other samples (Epstein et al. 1973; Kint and De Weert 1978; Kemmer et al. 1988). Occasionally irregular clumps of dense amorphous material have been described in perivascular deposits (Epstein et al. 1973). In our patients this excess material appeared mostly as a uniform band between outermost basal laminae and pericytes and it had a denser structure than fine granular material substance between basal laminae.

Our results of DIF are in accordance with that of three earlier series, in which a homogenous deposit of IgG in the vessel walls of the hand skin has been demonstrated in all 54 patients with PCT. In contrast, the presence of other immunoglobulins and complement has varied in different studies (Epstein et al. 1973; Kemmer et al. 1988; Vasil and Magro 2007). In an additional series including 16 patients with PCT, IgM (9/16) and C3 (11/16) could be demonstrated in the vessel walls of the hand skin in the majority of the cases, but IgG was present only in 44% of them (7/16) (Maynard and Peters 1992). Each sample, however, was positive for a deposit of one of the immunoglobulins with or without complement. In the same study IgG was expressed in the epidermal basement membrane in the minority of the cases (4/16), while C3 was present more frequently (9/16). Weak IgG deposit in the epidermal basement membrane of the dorsal side of hand has been demonstrated in 60-95% of cases of PCT in previous series (Epstein et al. 1973; Kemmer et al. 1988; Vasil and Magro 2007) but it could be demonstrated only in one of our four patients' samples. The variation in the results of direct immunofluorescense studies advocates the theory that occurrence of immunoglobulins and complement in the epidermal basement membrane and the vessel walls is an unspecific phenomenon and according to our results does not correlate with clinical remission.

The vessels in the non-exposed skin (the gluteal area) in our series were normal in thickness indicating that the repeated solar exposure is mandatory for developing of skin changes. This hypothesis is supported by the fact that immunoglobulin depositions were an uncommon finding in the non-exposed skin. In an earlier study the vessels of the normal skin in the medial arm have been normal in thickness (Epstein et al. 1973). The results of the direct immunofluorescencic studies have been negative in the majority of these cases, although electron microscopic studies have revealed mild changes in the basement membrane zone. Because the arms may have not been completely sun-protected, the electron microscopic findings suggest that vascular changes can be subclinical after minor sun exposure.

The preceding symptomatic phase at the time of biopsy was an average of 11 months in our study (range 3-24 months) and 5,2 years in the earlier study including 22 patients with active PCT (Epstein et al. 1973). Because the histopathological changes were comparable in both series, duration of the overt disease appeared to have no significant influence on the vascular changes. Moreover, the vascular changes arise already at the early stage of the clinically manifest disease. The duration of period during which the level of circulating uroporphyrin is elevated before the disease become manifest has been unclear. Possibly, the microscopical changes evolve concomitantly with the rise of the porphyrin levels because of developing hepatopathy and the sun exposure similarly as hypothesised in asymptomatic patients with VP (Article I). The development of vascular alterations may be a subclinical process beginning in the early stage of phototoxic damage which leads by additional currently unknown mechanisms to the cleavage of the epidermal basement membrane.

In the repeated biopsies taken in remission in patients with PCT, the vascular changes studied by LM remained similar to those seen before treatment in all patients except in one, in whom the vascular thickening was reduced. No improvement could be found in ultrastructural studies either. The duration of remission (6 vs. 12 months) had no effect on the histological results (Figs 3-4, VI). Thus, the clinical improvement of the skin symptoms and the biochemical remission had no significant effect on the histopathological changes in PCT during the short-term follow-up**.** The results indicate that the thickening of the vessel walls is chronic, possibly permanent phenomenon and does not directly correlate with clinical and biochemical activity of the disease.

Our findings are in accordance with a histopathological study performed in post-phlebotomy remission of patients with PCT. Remission of the disease had lasted from seven months to six years (Epstein et al. 1973). In that series, which included seven patients, thickening of the vessel walls could be demonstrated by LM in the dorsal side of the hand in five cases. These changes were milder when compared to those in an active PCT. In addition, weak deposition of IgG in the vessel walls could be demonstrated in the majority of the patients in remission (7/10) (Epstein et al. 1973). Although no repeated biopsies have been analysed during the follow-up of these patients, their and our results suggest that vascular changes are long-lasting after acute phase.

4.4. Comparison of the histopathological changes of the skin in VP, EPP and PCT

If our results obtained by different histopathological methods are evaluated as a group, the most prominent changes in EPP, VP and PCT are identified in the superficial dermal vessels walls (Table 28) suggesting that the vessels are prone to the photodamage in the skin. By LM thickening of the vessel walls is the major change in all porphyria types, and it is best demonstrated by PAS-staining. The most severe alterations were detected in EPP indicating that the phototoxic reaction is the most powerful in this patient group. The vascular changes were evident in the papillary dermis, but in EPP the changes were present more frequently in the superficial part of papillary dermis also than in VP and PCT. EPP was characterised by amorphous perivascular deposits extending also to the extravascular space, but no such deposits could be demonstrated in VP and PCT. This was the most striking difference between EPP and other porphyrias. Consequently, the light microscopic examination distinguishes clearly EPP from VP and PCT, but the changes found in VP and PCT are indistinguishable from each other corresponding to the similar clinical manifestations despite different mixture of circulating porphyrins (Poh-Fitzpatrick 1980). The absence of the inflammatory changes was a common feature in each subtype of porphyria.

The fine structure of the vessel wall was similar in VP and PCT consisting of the multilayered basement membrane and excess of fine granular substance between the layers, which were surrounded by the band of homogenous material. EPP was characterised by massive amorphous deposits outside the vessel walls in addition to similar alterations in the vessel walls as in VP and PCT. The ultrastructural findings were in accordance with the results obtained by LM and confirmed that the morphological changes in EPP differ clearly from those in VP and PCT and are the most severe of them.

Table 28. Histopathological changes of dermal vessel walls of the sun-exposed skin in patients with EPP, VP and PCT

	Dermal vessel wall					
Type of porphyria	LM		EM		DIF[1]	
	Exposed skin	*Protected skin*	*Exposed skin*	*Protected skin*	*Exposed skin*	*Protected skin*
EPP	Thickening Amorphous deposits	Normal	Multilayering of basal laminae Broad belt of amorphous material Amorphous deposits	Normal	IgG, IgA, IgM, C3	Negative
VP	Thickening	Thickening	Multilayering of basal laminae Belt of amorphous material	Multilayering of basal laminae	IgG, (IgA, IgM, C3)	(IgG, IgA, IgM, C3)
PCT	Thickening	Normal	Multilayering of basal laminae Belt of amorphous material	Normal	IgG, (IgA, C3)	(IgG)

[1](IgG), (IgA), (IgM), (C3)<50% of the patients studied

In the DIF study, the constant finding was the homogenous IgG depositions in the vessel walls in each type of porphyria. IgA, IgM and C3 were present more frequently in EPP than in PCT and VP. This was probably related to the more severe vascular damage in EPP resulting in an abundant leakage of immunoreactants from circulation. Almost identical findings suggest a common pathogenetic mechanism, which underlie the damage in the dermal vessels. On the whole our results are in accordance with the earlier studies, in which the regular presence of immunoreactants in the vessel walls has been demonstrated in all types of cutaneous porphyrias (Epstein et al. 1973; Maynard and Peters 1992).

Vascular changes of the exposed skin were observed in each type of porphyria also in the patients with no current skin symptoms suggesting that vascular changes are not directly correlated to the contemporary clinical condition and they are chronic in character. The follow-up of symptom-free VP patients suggests that the presence of microscopic changes does not predict the development of skin fragility later.

A mild thickening of the vessel walls could be demonstrated in the sun-protected skin of the patients with VP in contrast to the patients with EPP and PCT in whom thickness of the vessel walls was normal. The difference between PCT and VP might be related to a shorter duration of the disease in PCT, which usually manifests later in the life span compared to VP. In EPP the duration of the disease seemed to have no effect on the non-exposed skin, because the adult patients who had had symptoms since early childhood had normal vessels in the sun-protected skin.

The epidermal basement membrane of the sun-exposed skin showed no notable histopathological abnormalities outside the blister and erosion except a local reduplication of lamina densa close to blister in VP and PCT. Hyperkeratosis, hypergranulosis and acanthosis in the epidermis and a mild thickening of the epidermal basement membrane have been observed in series of samples of patients with EPP, VP and PCT (Epstein et al. 1973). No such changes, however, could be demonstrated in our study or in another study including patient samples with various cutaneous porphyrias (Maynard and Peters 1992).

The blisters studied in our patients with VP and PCT had a subepidermal localization as described in other patients (Dabski and Beutner 1991; Maynard and Peters 1992). In electron microscopic studies the blisters situated under the basal lamina in all our samples. In previous studies the blister formation in PCT has also been identified above lamina densa (Klein et al. 1983; Nagato et al. 1987). It has been postulated that the initial damage occurs at the level of lamina lucida and mechanical stimulus results in a damage under the basal lamina (Klein et al. 1983; Nagato et al. 1987).

The microscopical changes found in our studies are not specific for porphyrias. Similar thickening of the vessel walls and by DIF homogenous mantles of IgG and C5b-9 has been demonstrated in skin manifestations of diabetes mellitus and PCT (Vasil and Magro 2007). Electron microscopic studies of the gluteal skin of the patients with diabetes mellitus or photoaged skin have revealed multilayering of the basement membrane of the vessel walls surrounded by excess basement membrane like material mimicking vascular changes in porphyria (Braverman and Keh-Yen 1984). DIF testing is useful in differentiating VP and PCT from autoimmune subepidermal blistering diseases which are characterised by the presence of linear IgG and/or C3 at the epidermal basement membrane zone and usually the presence of inflammatory cells in the blistering area (Maynard and Peters 1992; Mutasim and Adams 2001). Classic type of epidermolysis bullosa acquisita, which clinically mimics PCT and VP presenting skin fragility, blistering, scarring and milia formation on the backs of the hands, is characterised by noninflammatory subepidermal blister formation as in PCT and VP. Direct immunofluorescence shows linear IgG and immunohistochemical staining type VII collagen in the epidermal basement membrane, but in the vessel walls can not been demonstrated immunoglobulins (Mutasim and Adams 2001). Porphyria is the only blistering disease in which homogenous deposits of immunoglobulins and complements can be demonstrated in the superficial dermal vessel walls. In pseudoporphyria clinical, histopathological and immunohistological changes are comparable to those found in PCT and VP (Maynard and Peters 1992). In that case porphyrin analyses from plasma, urine and faeces are normal and crucial to exclude a true porphyria.

The diagnosis of cutaneous porphyrias should always be based on the biochemical analyses only. Because taking a skin biopsy is an invasive procedure and represents a risk for scar formation and delayed wound closure especially in patients with cutaneous porphyrias who are prone to scarring after trauma, the non-invasive biochemical analyses should always be carried out first. The histopathological examination of the skin can provide supplementary information in the diagnostics and differential diagnostics of cutaneous porphyrias, but is not necessary for identification of porphyria.

CONCLUSIONS

- In 2006 156 Finnish patients with VP have been diagnosed since the 1960's, and to date 108 of them are alive. The estimated prevalence of VP is 2.1:100 000 and the incidence 0.2:1 000 000 (per year).
- In 2006 85 Finnish patients with PCT have been diagnosed, and to date 63 of them are alive. The prevalence is 1.2:100 000 and the incidence 0.5:1 000 000 (per year). Based on measurements of the UROD activity in patients' erythrocytes, 49% of patients with PCT has the familial form type II.
- In 2006 48 Finnish patients with EPP have been diagnosed, and 39 of them are alive. The prevalence is 0.8:100 000 and the incidence 0.1:1 000 000 (per year).
- Of the mutation carriers 37% have a manifest disease in VP. Frequency of skin symptoms has been stable during the follow-up, but acute attacks have become infrequent (51% vs. 18%) during the last two decades. Of the mutation carriers 30% manifest with skin symptoms and they represent currently 80% of symptomatic patients.
- In the patients with VP the major skin symptom was fragility (95%) of the skin in the backs of the hands. Blisters occurred in less than half of the patients.
- The patient with homozygous VP is a different entity with severe chronic skin lesions and demyelinating neuropathy without acute attacks. The I12T mutation could be detected in both of his alleles in the PPOX gene. In his family members at the heterozygous state no skin lesions or acute attacks could be detected.
- Transient correction of porphyrin metabolism by eight haem arginate infusions did not alleviate skin symptoms in four patients with VP except in one patient whose skin lesions disappeared transiently.
- In the patients with EPP the major skin symptoms were burning pain (96%) and swelling (92%) of the skin immediately after the sun exposure. The severity of photosensitivity correlated with the level of erythrocyte protoporphyrin concentration. More than a 50-fold increase predicted severe skin symptoms but manifestations were clearly affected by sun exposure. Hepatopathy appeared only in one patient.
- Four mutations (751delGAGAA, 1122delT, C286T, C343T) were characterised in four of 15 families with EPP.
- In the patients with PCT blistering (95%) and fragility (93%) of the skin in the backs of the hands occurred equally. The majority of the patients had one to three precipitating factors, of which alcohol was the commonest (78%). Hemochromatosis associated mutations were also frequent (50 %) but use of oestrogen (25%) and HCV or HBV infections (25%) were less common.
- In PCT, fatty liver disease was present in 48% of the 63 patients examined by imaging and was confirmed by liver biopsy in 67% of the 21 cases studied. Haemosiderosis was common (67%) in liver biopsy but no hepatoma could be found.
- In histopathological examinations studied in 20 patients with VP, eight patients with EPP and five patients with PCT, the major changes of the sun-exposed skin were thickening of the vessel walls of the upper dermis suggesting that this is the primary site of the phototoxic reaction in each type of porphyria. EPP was characterised by amorphous perivascular deposits also extending to the extravascular space, but no such deposits could be demonstrated in VP and PCT.

- The fine structure of the vessel wall was similar in VP and PCT consisting of the multilayered basement membrane and excess of finely granular substance between the layers, which were surrounded by the band of homogenous material. EPP was characterised by massive amorphous deposits outside of the vessel walls in addition to similar alterations in the vessel walls as in VP and PCT.
- In the DIF study, the constant finding was the homogenous IgG deposits in the vessel walls in each type of porphyria. IgA, IgM and C3 were present more frequently in EPP than in PCT and VP suggesting that the most severe vascular damage in EPP causes an abundant leakage of immunoreactants from the circulation.
- In VP, the microscopic changes were not predictive for later skin manifestations.
- In PCT, no remarkable improvement of vascular changes was found during clinical and biochemical remission indicating that microscopic changes are irreversible.
- The excess material in and around vessel walls consists of immunoglobulins and other proteins derived from the circulation in addition to basement membrane constituents produced locally by fibroblasts. These finding suggest that thickening of the vessel wall is a result of the repeated damage and the repairing process in the vessel wall.

ACKNOWLEDGEMENTS

This work was carried out at the Departments of Medicine and Dermatology, University of Helsinki, Finland, during 1987-2008. Many Heads of the Department of Dermatology are thanked for providing me an opportunity and inspiring work atmosphere throughout these years. I want to express my gratitude particularly to Professor, emeritus Kimmo Mustakallio, the previous Head of the Department of Dermatology for his support at the early phase of my study, and Professor Reijo Tilvis, the previous Head of Institute of Clinical Medicine for excellent research facilities in Meilahti Hospital and Biomedicum-Helsinki. I am very grateful to the Heads of the Department of Dermatology, Professor Timo Reunala and Professor Annamari Ranki, for the chance to work in the clinic and their long-lasting belief in me finalizing my thesis.

My special thanks are to Docent Kirsti-Maria Niemi, my supervisor, because she was first to suggest the histopathological study of porphyrias to me. She introduced me to the world of light and electron microscopy. Her intelligence, vast knowledge of dermatopathology and uncompromising attitude towards scientific work has been admirable.

I express my warmest thanks to Professor Pertti Mustajoki, the supervisor for the first ten years, for guiding me to the secrets of metabolism and clinical manifestations of porphyrias and an opportunity to utilize his unique and internationally recognized patient material. His enthusiasm was encouraging and his warm personality supported me on this zigzag way.

Most of all I am indebted to Docent Raili Kauppinen, my supervisor during the last 15 years. She has guided me in interpreting the results of molecular genetics and she has been a valuable source of information in various aspects of medicine in addition to porphyrias. Her never-ending energy, vivid personality and persistence have supported me throughout these -sometimes even desperate - years and she has not let me lay down the whole work. I am very grateful for her patience and readiness whenever to discuss about research and topics extending beyond the everyday work. Fortunately, we have had a sense of humour during this long journey!

I thank warmly Docent Arja-Leena Kariniemi, my co-author, who supported me enormously especially by teaching dermatopathology. Her valuable comments during writing the manuscript clarified my ideas of porphyria and her optimistic attitude helped me to endure to the end. We have shared many delightful times travelling in a congress-group and learned also new aspects of life outside dermatology.

I am very grateful to Professor Aarne Oikarinen for his careful dissection of my manuscript and valuable comments to improve the final version, and for his encouraging attitude to this work. I appreciate Professor Jorge Frank who is known for his special interest in inherited skin diseases such as porphyrias, his positive attitude to my thesis and important comments. I would like thank Dr Yevgeny Pavlushkov (MD, Master of Philosophy) for his careful examination of my English in the manuscript.

I am deeply grateful to Professor Raimo Tenhunen, the previous Head of the Department of Clinical Chemistry for the kindness providing me excellent service in his biochemical laboratory. His knowledge of haem metabolism was enormous help and his friendly encouragement supported me all the time. The positive atmosphere and extremely skilful staff in his laboratory have made my study easy to perform. I thank warmly his personal including laboratory nurses Tarja Andersson and Aila Åkerlund and chemist Eila Laitinen for their competent assistance with biochemical analyses and friendship. I wish to thank chemist Sirpa Ranta who has joined our “porphyria family” during the recent years, for her proficiency, enthusiasism to porphyria and helpfulness.

I am very grateful to MSc Helena Halme, the earlier chemist in Department of Dermatology. She was interested particularly in biochemistry of porphyrias and provided me an invaluable

help at my first steps in the fields of porphyria.

I have become familiar with the scientific laboratories in Meilahti Hospital and in Biomedicum. Pipeting was not completely impossible for me in the guidance of our skilful bioanalysists Hannele Pihlaja, Helena Ahola and Susanna Mehtälä who are warmly thanked.

Young scientists Dr Sami Mustajoki, Dr Mikael von und zu Fraunberg and Dr Elena Pischik in our Porphyria Reseach Group have done their thesis much faster than me. I am thankful for their pleasant company, inspiring scientific and more personal discussions and being a part of this group. Mikael has shared with me the studies related to VP and his statistical skills have been fantastic. Dr Matti Henriksson investigated molecular genetics in our EPP study and was a pleasant co-author. Elena has been worth gold since she has helped me with a computer finalizing the text frame and checked all the references and tables with the skills you do not learn in the clinic. Her brilliant intelligence not only in neurology and porphyria but in many fields of life and warm heart has lighted my days when we have got together.

I wish to thank Department of Dermatology for all the support which I have got from my colleagues, nurses and patients with porphyria during these 25 years. Dr Terttu-Liisa Ahokas and Professor, emerita Leena Rechardt who have been a part of our travelling-in-congresses-group, have reminded me about the life outside the scientific world and I have often followed their advice with pleasure.

I thank sincerely laboratory technicians Birgitta Arteva, Anna-Mari Hakulinen and Liisa Sundman for their excellent technical assistance with preparing skin samples for electron microscopy and immunohistochemistry.

I thank warmly Ralf Baumann, Raili's son, who provided light to the cover design and inside of my thesis with his skilful pictures. I express my kindly thanks to his father, Marc who has been a funny and encouraging company during the lunch breaks and has not hesitated to help whenever our computer has tilted.

I am grateful to the colleagues in the Departments of Dermatology in Central Hospitals throughout Finland for providing patients with cutaneous porphyria to this stydy.

Finally, I wish to thank my mother and sisters for their understanding on this long way. I owe my heartfelt thanks to Matti for his love, endless patience, sense of humour and careful housekeeping during these years.

This study was financially supported by the grants of Finnish Cultural Foundation, the Sigrid Juselius Foundation, the Paolo Foundation, the Academy of Finland, the Research Funds and Clinical Research Institute of Helsinki University Central Hospital, and the University of Helsinki.

Helsinki, January 2009

Kaisa Timonen

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Appendix 1: Biochemical diagnosis of hepatic cutaneous porphyrias in symptomatic and symptom-free patients

	VP		**PCT**		**HCP**	
Porphyrin analyses	Symptomatic	Asymptomatic or clinically in remission	Symptomatic	Clinically and biochemically in remission	Symptomatic	Asymptomatic or clinically in remission
Plasma fluorescence	626 nm	626 nm or negative	617-619 nm	Negative	617-619 nm	617-619 nm or negative
Erythrocyte porphyrins	Normal	Normal	Normal	Normal	Normal	Normal
Urine porphyrins	Coproporphyrin increased or normal	Coproporphyrin increased or normal	Uroporphyrin and 7-Carboxyl porphyrin increased	Uroporphyrin and 7- Carboxyl porphyrin slightly increased or normal	Coproporphyrin III increased	Coproporphyrin III increased or normal
Faecal porphyrins	Protoporphyrin increased	Protoporphyrin increased or normal	Coproporphyrin increased or normal	Normal	Coproporphyrin III increased	Coproporphyrin III increased or normal
Enzyme analysis	Ly-PPOX decreased	Ly-PPOX decreased	E-UROD Type I normal Type III normal Type II decreased	E-UROD Type I and III: normal Type II decreased	Coproporphyrinogen oxidase decreased	Coproporphyrinogen oxidase decreased
DNA test if a mutation in a family is known.	Positive	Positive	Type II and III Positive	Type II and III Positive	Positive	Positive

Appendix 2: Biochemical diagnosis of erythropoietic porphyrias

	EPP		CEP
Porphyrin analyses	Symptomatic or clinically in remission	Never symptoms	Symptomatic
Plasma Fluorescence	634 nm	Negative	617-619 nm
Erythrocyte porphyrins	Protoporphyrin (free) increased	Protoporphyrin normal or slightly increased	Uroporphyrin I, coproporphyrin I and protoporphyrin increased
Urine porphyrins	Normal	Normal	Uroporphyrin I and coproporphyrin I increased
Faeal porphyrins	Protoporphyrin increased or normal	Normal	Coproporphyrin I and protoporphyrin increased
Enzyme analysis	E-FECH decreased	E-FECH decreased	Uroporphyrinogen III synthase decreased
DNA test if a mutation in a family is known.	Positive	Positive	Positive

Appendix 3: Differential diagnosis of cutaneous porphyrias according to the skin manifestations

Skin manifestation	*Differential diagnosis*	*Diagnostic method*
Photosensitivity	Polymorphous light eruption	Clinical signs and history Phototesting Histopathology (if necessary)
	Solar urticaria	Clinical signs and history Phototesting
	Lupus erythematosus	Clinical signs and history Histopathology, DIF[1] Lupus serology
	Photocontact dermatitis	Clinical signs and history Phototesting Histopathology (if necessary)
Oedema	Angioedema	Clinical signs and history Serum complement analysis (HAE)
Erythema	Erythema solare (Sunburn)	Clinical signs and history Negative porphyrin biochemistry Histopathology (if necessary)
Burning sensation of the skin		
	Erythema solare (Sunburn)	As above
	Erythromelalgia	Clinical signs and history Heat provocation
	Brachioradial pruritus	Clinical signs and history
Blister formation and skin fragility on the sun-exposed areas		
	Pseudoporphyria	Histopathology, DIF Negative porphyrin biochemistry
	Epidermolysis bullosa acquisita	Histopathology, DIF
	classic type	Sodium chloride split skin+DIF Autoantibodies to collagen VII
	Epidermolysis bullosa	Histopathology, DIF (negative) Electron microscopy Mutation analysis Clinical signs and history Family history
	Bullous amyloidosis	Histopathology, DIF (negative) Clinical signs and history
Blister formation without skin fragility on the sun-exposed areas		
	Bullous pemphigoid	Histopathology, DIF Autoantibodies to EBMZ[2] antigens Clinical signs and history
	Bullous dermatitis of diabetes	Histopathology, DIF (negative) Clinical signs and history
	Phytophotodermatitis	Histopathology, DIF (negative) Clinical signs and history
	Bullous lupus erythematosus	Histopathology, DIF, Lupus serology Clinical signs and history
Hypertrichosis	Hirsutismus	Endocrinological examination
Hyperpigmentation	Melasma, systemic diseases, phototoxic reaction	Clinical and laboratory examination Histopathology (if necessary)

[1]DIF = Direct immunofluorescence [2]EBMZ = Epidermal basement membrane zone

Appendix 4: Differential diagnosis of cutaneous porphyrias according to the histopathological and immunofluorescence changes

Histopathological finding	*Diagnosis*	*Direct imunofluorescence of the skin*
Subepidermal bulla		
Noninflammatory	PCT, VP	IgG±IgA, IgM, C3 in the vessel walls and variably at EBMZ[1]
Noninflammatory	Pseudoporphyria	IgG±IgA, IgM, C3 in the vessel walls and variably at EBMZ
Noninflammatory	Bullous dermatitis of diabetes	Negative
Noninflammatory	Bullous amyloidosis	Negative
Noninflammatory	Junctional epidermolysis bullosa	Negative
Noninflammatory or inflammatory	Epidermolysis bullosa acquisita classic or inflammatory type	Linear IgG±C3 at EBMZ
Eosinophilic infiltrate	Bullous pemphigoid	Linear IgG±C3 at EBMZ
Neutrophilic infiltrate	Dermatitis herpetiformis	Granular IgA at EBMZ
Neutrophilic infiltrate	Linear IgA disease	Linear IgA±C3 at EBMZ
Lymphocytic infiltrate or noninflammatory	Bullous lupus erythematosus	Subepidermal band of IgG, IgM, C3
Thickening of the dermal vessel walls		
PAS-positive Amorphous deposits	EPP	IgG±IgA,IgM,C3 in the vessel walls
PAS-positive	PCT, VP	IgG±IgA,IgM,C3 in the vessel walls and variably at EBMZ
PAS-positive	Diabetes	Negative
PAS-positive	Rheumatoid arthritis	Negative
Congo-positive	Amyloidosis	Negative

[1]EBMZ = Epidermal basement membrane zone